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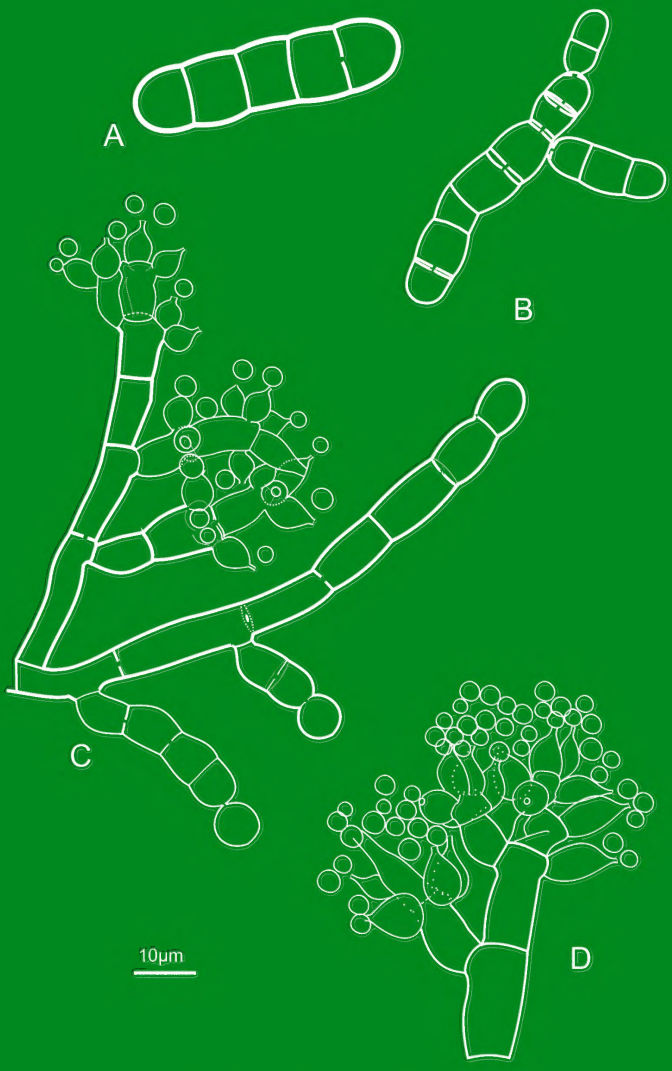

Tretolylea pleiomorpha sp. nov.
(Cantillo-Pérez, Gusmão & Castañeda-Ruiz— FIG. 1, p. 979)
TAIMY CANTILLO, *artist*

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Reviewers — volume one hundred thirty (4)

The Editors express their appreciation to the following individuals who have, prior to acceptance for publication, reviewed one or more of the papers prepared for this quarter.

Joe Ammirati
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Merlin White
Lidia Yakovchenko
Ming Ye
Xiu-Guo Zhang

Errata from previous volumes

Volume 125

p.305, 9th line from bottom *[Mattirolia ohiensis]*	FOR: 154	READ: 155
p.305, 8th line from bottom [*Mattirolia platensis*]	FOR: p. 154	READ: p. 155
p.306, 4th line from bottom [*Setosynnema yunnanense*]	FOR: p. 22	READ: p. 82

Volume 126

p.249, lines 5 & 6 [*Ambomucor*]	FOR: *seratioinflatus* READ: *seratoinflatus*

Volume 127

p.233, lines 17 & 18 [*Helicodochium*]	FOR: p. 5	READ: p. 6
p.233, 5th line from bottom	FOR: p. 154	READ: p. 146

Volume 130(2)

Validation of the name *Matsushimiella paraensis*

Josiane Santana Monteiro, Luís Fernando Pascholati Gusmão & Rafael F. Castañeda-Ruiz

The proposed name "*Matsushimiella paraensis* J.S. Monteiro, Gusmão & R.F. Castañeda, sp. nov." on page 312 of Mycotaxon 130(2) was not validly published because the cited registration number, "MB 807635", was incorrect and is assigned to a separate name. Art. 42.1 of the International Code of Nomenclature (McNeill & al. 2012) requires that after January 1, 2013, 'the citation in the protologue of the identifier issued by a recognized repository for the name (Art. 42.3) is an additional requirement for valid publication.' We hereby validate the name *Matsushimiella paraensis* J.S. Monteiro, Gusmão & R.F. Castañeda, sp. nov. with the assigned number MB 810689, with its description published in Mycotaxon 130: 312. 2015 and its holotype designated as HUEFS 210425. Additional details were published in the original publication.

From the Editor-in-Chief

Fungal identification numbers redux — The IBC permanent Nomenclature Committee for Fungi is currently engaged in a spirited discussion as to whether citing an incorrect fungal identification number after a newly proposed name should render that new name invalid. There is no question as to whether the current International Code of Nomenclature for algae, fungi, and plants in fact does specify that a name published with the wrong number is an invalidly published name. Article 42.1 requires "the citation in the protologue of the identifier issued," and leaves no apparent room to correct mistakes. Therefore, until the Melbourne Code is amended to regard a typographical slip of the pen producing an incorrect number as a 'correctable' error (as is now permitted for epithets misspelled by authors—most frequently by those ignorant of the grammatical rules of Latin gender agreement), FungalName, IndexFungorum, and MycoBank identification numbers must be presented with the new names exactly as they have been issued by the repository.

The 'new' fungal identification numbers are important enough that we again ask all our authors to make certain that the numbers match the names for which they have been issued. Mycotaxon also now includes them in the list of nomenclatural novelties and typifications that closes each issue. Correctly, we hope!

Mycotaxon 130(4) contains 29 papers (+ links to 4 new regional mycobiotas) by 118 authors representing 24 countries and revised by 57 expert reviewers.

Within its pages are one new genus (*Tetrolylea* from Brazil) and 21 species new to science representing *Acaulospora, Codinaea, Dictyosporium, Pyramidospora, Synchaetomella, Tetrolylea,* and *Trichoconis* from Brazil; *Entyloma* from Israel; *Hymenoscyphus, Kroswia, Phyllachora,* and *Terriera* from China; *Lecanicillium* from Korea; *Neoalbatrellus* from western Canada and the USA; *Phoma* from Turkey; *Pouzarella* from the USA; and *Tuber* from Serbia.

In addition to range extensions and/or new hosts for previously named taxa, we also offer one new combination in *Codinaea, Gnomoniopsis smithogilvyi* as the correct name for the chestnut pathogen, a complete list of the species currently accepted in *Alternaria* sect. *Alternaria,* a monographic treatment of *Kroswia* in Korea, a multigene phylogeny of *Hymenoscyphus,* the DNA bar-code assisted identification of three *Pisolithus* species in Macedonia, an overview of myxomycetes in Chihuahua Desert, a pictorial key to *Dictyosporium* conidia, and a revised key to the American and European species of *Scutiger* sensu lato.

With our very best wishes for a joyful and productive 2016,

Lorelei L. Norvell (*Editor-in-Chief*)
29 December 2015

publication date for volume one hundred thirty (3)
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Four steps to successful Mycotaxon publication in 2016

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3—Final submission: Consult experts, revise and thoroughly proof manuscripts, and prepare error-free text and image files ready for immediate publication. Label the Email with the accession number before attaching the (i) 2016 Mycotaxon submission form; (ii) text files for main text, tables, and legends; and (iii) final art files to the *Editor-in-Chief* <editor@mycotaxon.com>. The *Editor-in-Chief* usually Emails all coauthors and expert reviewers within two weeks of final submission, but please wait at least 14 days before sending a follow-up query (without attachments).

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The chestnut pathogen *Gnomoniopsis smithogilvyi* (*Gnomoniaceae, Diaporthales*) and its synonyms

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Abstract — Two species, *Gnomoniopsis smithogilvyi* and *G. castaneae*, were described independently in 2012 as causal agents of chestnut (*Castanea*) rot in Australasia and Europe. A comparative morphological analysis and five-marker phylogenetic analysis of ITS, TEF1-α, β-tubulin, MS204, and FG1093 confirm that both names refer to the same species, and an investigation of their exact publication dates demonstrates that *G. smithogilvyi* has priority over *G. castaneae.*

Key words — canker, endophyte, *Gnomonia*, nut rot

Introduction

Species of *Gnomoniopsis* have a mainly northern hemisphere distribution and are economically important pathogens of the rosaceous hosts blackberry, raspberry, and strawberry (Bolay 1971, Monod 1983, Maas 1998, Sogonov et al. 2008, Walker et al. 2010).

Shuttleworth et al. (2012a) described a new species, *Gnomoniopsis smithogilvyi,* as the causal agent of chestnut rot, as an endophyte of reproductive and vegetative tissues, and as a saprobe of European chestnut (*Castanea sativa* Mill.) and hybrids of Japanese chestnut and European chestnut (*Castanea crenata* Siebold & Zucc. × *C. sativa)* in southeastern Australia (Shuttleworth et al. 2012a,b). Shuttleworth et al. (2012a) also found *G. smithogilvyi* occurring in New Zealand on *C. crenata, C. sativa,* and *Castanea* sp. and associated with *C. sativa* in India and Italy.

Visentin et al. (2012) described a new species, *Gnomoniopsis castaneae* [as "*castanea*"], as the causal agent of nut rot, endophyte, and saprobe of *C. sativa* in France, Italy, New Zealand, and Switzerland. They referred to the fungus reported earlier as a *Gnomonia* sp. (informally named "*G. pascoe*") as the main causal agent of chestnut rot in Australia and New Zealand (Smith & Ogilvy 2008). Sequences from Swiss isolates have been deposited as *G. castaneae* (Dennert et al. 2014), and an Italian isolate from necrosis of insect-induced galls of *Castanea* has also been identified as *G. castaneae* (Vinale et al. 2014).

The aim of this paper is to clarify the taxonomic relationship of *G. smithogilvyi* and *G. castaneae* and, if they are conspecific, to determine which name has priority.

Materials & methods

Morphology

Observation of cultures of *G. smithogilvyi* CBS 130190 (ex-type) from Australia, *G. castaneae* MUT 401 (ex-type) from Italy, and "*Gnomonia pascoe*" MUT 411 from New Zealand were made from herbarium material. Herbarium specimens of the sexual phase were not examined in this study.

Sequencing and phylogenetic analyses

Fragments of four protein coding markers TEF1-α, β-tubulin, MS204, FG1093 were amplified and sequenced for *Gnomoniopsis smithogilvyi* CBS 130190 (ex-type), CBS 130188 and CBS 130189, *G. castaneae* MUT 401 (ex-type) and "*Gnomonia pascoe*" MUT 411 using primer pairs TEF1-α: EF1-728F (Carbone & Kohn 1999)/EF1-1567 (Rehner 2001), β-tubulin: T1/T2 (O'Donnell & Cigelnik 1997), MS204: amplification E1F1/E5R1, sequencing E3F1/E4R1a/E5R1a, FG1093: ElFla/E3Rla (all MS204 and FG1093 primers; Walker et al. 2012).

Thermocycling conditions were identical to Walker et al. (2010, 2012). In addition, ITS sequences and reference sequences were downloaded from GenBank (Table 1). Alignments were completed with the MAFFT alignment option (Katoh et al. 2002) of Geneious® R7 v7.1.2 (Biomatters Ltd., New Zealand) with default settings applied. Six alignments were completed, one for each of the five individual marker datasets and one for the combined five-marker dataset.

For the TEF1-α, β-tubulin, MS204, FG1093 and the combined five-marker dataset, nineteen isolates were selected including reference species of *Gnomoniopsis*. The ITS analyses included an addition four isolates from India, six from Italy, two from France, six from New Zealand, and two from Switzerland. Sequences from studies in India and Switzerland associated with cankers of *C. sativa* were also included (Dar & Rai 2013, 2015, Pasche et al. 2015).

Two outgroup isolates were selected based on Walker et al. (2012), specifically *Ophiognomonia setacea* CBS 128354 and *Plagiostoma* sp. CBS 128351. *Gnomoniopsis guttulata* was excluded from the five-marker dataset because sequences for four of the five markers were not available. Although MS204 and FG1093 sequences were not available for *G. racemula,* this taxon was included in the five-marker dataset.

Table 1. GenBank accessions and country of collection for *Gnomoniopsis* and outgroup isolates used in the phylogenetic analyses to delineate *G. smithogilvyi* and its synonyms.

Species	Isolate	Country	GenBank accessions				
			ITS	TEF1-α	β-tubulin	MS204	FG1093
G. alderdunensis	CBS 125680	U.S.A.	GU320825	GU320801	GU320787	JF319036	JF274653
G. chamaemori	CBS 804.79	Finland	GU320817	GU320809	GU320777	JF319029	JF274646
G. clavulata	CBS 121255	U.S.A.	EU254818	GU320807	EU219211	JF319027	JF274644
G. comari	CBS 806.79	Finland	EU254821	GU320810	EU219156	JF319030	JF274647
G. fructicola	CBS 208.34	France	EU254826	GU320808	EU219149	JF319028	JF274645
G. guttulata	MS 0312	Bulgaria	EU254812	–	–	–	–
G. idaeicola	CBS 125676	U.S.A.	GU320821	GU320811	GU320784	JF319037	JF274654
G. macounii	CBS 121468	U.S.A.	EU254762	GU320804	EU219126	JF319024	JF274641
G. occulta	CBS 125678	U.S.A.	GU320829	GU320800	GU320786	JF319033	JF274650
G. paraclavulata	CBS 123202	U.S.A.	GU320830	GU320815	GU320775	JF319025	JF274642
G. racemula	CBS 121469	U.S.A.	EU254841	GU320803	EU219125	–	–
G. sanguisorbae	CBS 858.79	Switzerland	GU320818	GU320805	GU320790	JF319031	JF274648
G. smithogilvyi	CBS 130190	Australia	JQ910642	**KR072534**	JQ910639	**KR072539**	**KR072544**
	CBS 130189	Australia	JQ910644	**KR072535**	JQ910641	**KR072540**	**KR072545**
	CBS 130188	Australia	JQ910643	**KR072536**	JQ910640	**KR072541**	**KR072546**
	LSV908	France	KJ173703	—	—	—	—
	MUT 815	France	JQ898298	NU	—	—	—
	Gn_TN5	Italy	JX094888	—	—	—	—
	MUT 401	Italy	HM142946	**KR072537**	**KR072532**	**KR072542**	**KR072547**
	M4perDA	Italy	JN793531	—	—	—	—
	M3Cu	Italy	JN793534	—	—	—	—

Concluded on p. 932

Table 1, concluded

Species	Isolate	Country	GenBank accessions				
			ITS	TEF1-α	β-tubulin	MS204	FG1093
(*G. smithogilvyi*, continued)	M6EmA	Italy	JN793535	—	—	—	—
	Gm_TN1	Italy	JX094889	—	—	—	—
	Gm_TO	Italy	JX094892	—	—	—	—
	AU/DBT305	India	KC963938	—	—	—	—
	AU/DBT306	India	KC963940	—	—	—	—
	AU/DBT296	India	KC963944	—	—	—	—
	INDC(BSF5)A	India	JQ268073	—	—	—	—
	MUT 411	New Zealand	HM1142948	**KR072538**	**KR072533**	**KR072543**	**KR072548**
	ICMP:14492	New Zealand	KC145967	NU	—	—	—
	ICMP:14080	New Zealand	KC145870	NU	—	—	—
	CBS 121477	New Zealand	EU254854	—	—	—	—
	CBS 121917	New Zealand	EU254851	NU	—	—	—
	CBS 121918	New Zealand	EU254855	NU	NU	—	—
	MUT 810	Switzerland	JQ898295	NU	—	—	—
	Ge1	Switzerland	KP824754	NU	NU	—	—
G. tormentillae	CBS 904.79	Switzerland	EU254856	GU320795	EU219165	JF319032	JF274649
Ophiognomonia setacea	CBS 128354	U.S.A.	JF514847	JF514823	JF514839	JF319035	JF274652
Plagiostoma sp.	CBS 128351	U.S.A.	JF514852	JF514833	JF514836	JF319034	JF274651

One isolate per reference species was selected. GenBank accessions for sequences generated in the current study are in bold. AU and IND = isolates associated with Dar & Rai (2013); CBS = Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; Ge1 = isolate associated with Pasche et al. (2015); Gm and Gn = isolates associated with Maresi et al. (2013); ICMP = International Collection of Micro-organisms from Plants, Landcare Research, Auckland, New Zealand; MS = isolate associated with Sogonov et al. (2008); MUT = Mycotheca Universitatis Taurinensis, University of Turin, Italy. Other isolates downloaded as sequences from unpublished work.

Two criteria were used for phylogenetic species recognition: genealogical concordance phylogenetic species recognition (GCPSR; Taylor et al. 2000) and the genealogical sorting index (GSI; Cummings et al. 2008). A conditional comparison test to determine GCPSR was completed to identify support for the *G. smithogilvyi* clade on the individual marker datasets using maximum parsimony bootstrap support (MPBS) ≥70% (Mason-Gamer & Kellogg 1996, Kellogg et al. 1996). The GSI is a statistical measure to determine if predefined groups on a phylogenetic tree have reached reciprocal monophyly thus sharing exclusive ancestry. It is interpreted on a 0 to 1 continuum where 0 = lack of genealogical divergence from other groups and 1 = monophyly (Cummings et al. 2008). All five single marker and the combined marker alignments were independently tested with GSI using 100 randomly selected trees calculated from the GARLI maximum likelihood bootstrap (MLBS) tree distribution computed with the GARLI (v2.1) web service hosted at molecularevolution.org (Bazinet et al. 2014, Zwickl 2006). The GSI analyses were performed with a web service hosted at molecularevolution.org (GSI v0.92; Cummings et al. 2008) using 10,000 permutations to determine statistical significance (*P*-value ≤0.05). Independent GSI values from the MLBS tree distribution were pooled to calculate an ensemble statistic GSI_T for each marker and the combined marker alignment (Table 2).

Table 2. Phylogenetic species recognition of *Gnomoniopsis smithogilvyi*

Marker	GCPSR (MPBS %)	gsi_T	p-value
ITS	99	1	0.0001
TEF1-α	100	1	0.0001
β-tubulin	100	1	0.0005
MS204	100	1	0.0001
FG1093	100	1	0.0003
Concatenated dataset	100	1	0.0001

Maximum parsimony bootstrap support (MPBS) ≥70% indicates strong support for the *G. smithogilvyi* clade. The gene sorting index (GSI) is based on a 0 to 1 continuum, 0 = lack of genealogical divergence from other groups and 1 = monophyly; p-values ≤0.05 indicate the GSI is statistically significant.

Maximum parsimony (MP), maximum likelihood (ML), and Bayesian analyses were used to infer phylogenetic trees. MP and ML trees were inferred with MEGA6 (Tamura et al. 2013). The Bayesian trees were inferred with MRBAYES (Huelsenbeck & Ronquist 2001). The MP analyses used the heuristic search option with 1000 random sequence addition replicates, the tree-bisection reconnection (TBR) branch-swapping option with MULTTREES on, and MAXTREES set at 1000. All characters had equal weight. For the ML and Bayesian analyses, jModeltest 2.1 (Posada 2008) was used to determine the optimal model that best fit the individual marker and the five-marker datasets. The resulting model was GTR+I+G for all datasets.

Branch support in ML and MP analyses were determined with maximum likelihood bootstrap (MLBS) and maximum parsimony bootstrap (MPBS) analyses in MEGA6. The MLBS and MPBS were calculated using 1000 bootstrap replicates, with 10 random addition replicates per bootstrap replicate in the MPBS analysis (Felsenstein 1985). A threshold of ≥70% support was used as a cut-off for strongly supported tree nodes for both MPBS and MLBS. The Bayesian analyses used the Markov Chain Monte Carlo (MCMC) method. Identical nucleotide substitution models were used ML and Bayesian analyses. Four heated chains were run with 5 million generations, a subsampling frequency of 2000 generations and a burn-in of 500,000 generations (10%).

Results & discussion

Morphology

Measurements of asexual culture characters agreed with the published descriptions of Shuttleworth et al. (2012a) and Visentin et al. (2012). However, conidia of all isolates were hyaline and mostly multi-guttulate (although occasionally uni- and bi-guttulate). The differences in the published descriptions of the sexual states were the biseriate arrangement of ascospores in *G. smithogilvyi* (Shuttleworth et al. 2012a) and the uniseriate or irregularly multiseriate arrangement in *G. castaneae* (Visentin et al. 2012).

Morphologically, the sexual states of *Gnomoniopsis* spp. are characterised by small black perithecia that are immersed in host tissue, solitary, lacking a stroma or in groups aggregated in a minimal stroma, with a single central, marginal or lateral neck (Sogonov et al. 2008). Asci are oval to fusiform with a visible apical ring and contain eight spores. The ascospores are one-septate, oval to fusiform, and without appendages (except in *G. tormentillae;* Barr 1978). The known asexual states of *Gnomoniopsis* have subglobose pycnidia with one-celled hyaline conidia that are oval, oblong, globose or femur-shaped. *Gnomoniopsis smithogilvyi* fits morphologically into this description of *Gnomoniopsis.*

Gnomoniopsis smithogilvyi can be separated morphologically from its sister species *G. paraclavulata* Sogonov and *G. clavulata* (Ellis) Sogonov by having smaller ascospores (*G. smithogilvyi* mean length × width = 7 µm × 2 µm [(Shuttleworth et al. 2012a); *G. paraclavulata* 9.5 µm × 3.5 µm (Sogonov et al. 2008); *G. clavulata* 9 µm × 4 µm (Sogonov et al. 2008)].

Host range

Most *Gnomoniopsis* species show host specificity to genera in *Fagaceae*, *Onagraceae*, or *Rosaceae* (Walker et al. 2010). *Gnomoniopsis smithogilvyi*, *G. paraclavulata,* and *G. clavulata* can be separated by their known host ranges. All three species are reported on fagaceous hosts, but *Gnomoniopsis smithogilvyi* is reported only on *Castanea* spp., *G. paraclavulata* is reported only on *Quercus* spp., and *G. clavulata* is reported on *Fagus sylvatica* and *Quercus* spp. (Sogonov et al. 2008).

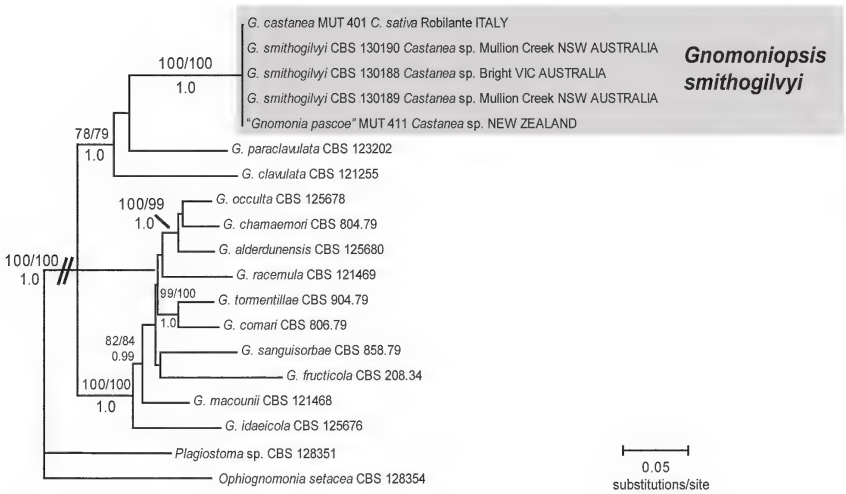

FIG. 1. ML phylogeny of combined ITS/TEF1-α/β-tubulin/MS204/FG1093 sequences of *G. smithogilvyi*, *G. castanea*, and "*G. pascoe*", with single isolates of *Gnomoniopsis* reference taxa and outgroup (ML score = –lnL score of 16532.47). Maximum likelihood bootstrap (MLBS), maximum parsimony bootstrap (MPBS) values ≥70%, and Bayesian posterior probabilities (PP) ≥0.95 are displayed at each branch in the order MLBS/MPBS/PP.

Phylogenetic analyses

The alignment lengths for the six datasets including gaps were ITS: 508 bp, TEF1-α: 917 bp, β-tubulin: 593 bp, MS204: 774 bp, FG1093: 351 bp, and the combined five-marker: 3137 bp. The topology of the ML five-marker phylogeny grouped *Gnomoniopsis* isolates from *Castanea* in Australia, Italy and New Zealand in the same strongly supported lineage (MLBS = 100%; FIG. 1). Of the 3137 total-character MP five-marker dataset, 763 were parsimony informative, 471 parsimony uninformative, and 1903 constant. The MP heuristic search produced three most parsimonious trees of 2717 steps (CI = 0.687523, RI = 0.701477, RCI = 0.482281 for all sites, and iCI = 0.601034, iRI = 0.701477, iRCI = 0.421611 for parsimony informative sites). The topology of the MP five-marker phylogeny was similar to the ML phylogeny, grouping *Gnomoniopsis* isolates from *Castanea* in Australia, Italy, and New Zealand in the same strongly supported lineage (MPBS = 100%). The most likely ML five-marker tree had –lnL score 16532.47. The Bayesian distribution of likelihood scores was –lnL 16403.508–16414.583, and the PP of the branch supporting the isolates from *Castanea* was 1.00.

The ML ITS analysis resulted in a most likely tree with a –lnL score of 1866.45. Twenty-five isolates from *Castanea* collected in Australia, France, India, Italy,

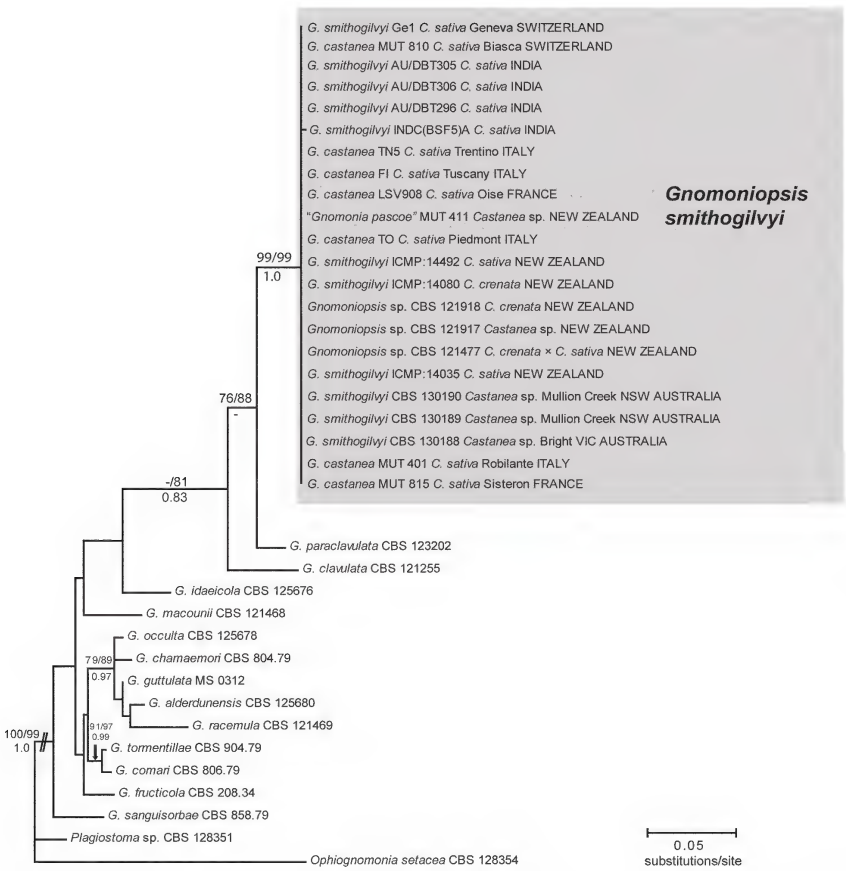
G. smithogilvyi Ge1 C. sativa Geneva SWITZERLAND
G. castanea MUT 810 C. sativa Biasca SWITZERLAND
G. smithogilvyi AU/DBT305 C. sativa INDIA
G. smithogilvyi AU/DBT306 C. sativa INDIA
G. smithogilvyi AU/DBT296 C. sativa INDIA
G. smithogilvyi INDC(BSF5)A C. sativa INDIA
G. castanea TN5 C. sativa Trentino ITALY
G. castanea FI C. sativa Tuscany ITALY
G. castanea LSV908 C. sativa Oise FRANCE
"Gnomonia pascoe" MUT 411 Castanea sp. NEW ZEALAND
G. castanea TO C. sativa Piedmont ITALY
G. smithogilvyi ICMP:14492 C. sativa NEW ZEALAND
G. smithogilvyi ICMP:14080 C. crenata NEW ZEALAND
Gnomoniopsis sp. CBS 121918 C. crenata NEW ZEALAND
Gnomoniopsis sp. CBS 121917 Castanea sp. NEW ZEALAND
Gnomoniopsis sp. CBS 121477 C. crenata × C. sativa NEW ZEALAND
G. smithogilvyi ICMP:14035 C. sativa NEW ZEALAND
G. smithogilvyi CBS 130190 Castanea sp. Mullion Creek NSW AUSTRALIA
G. smithogilvyi CBS 130189 Castanea sp. Mullion Creek NSW AUSTRALIA
G. smithogilvyi CBS 130188 Castanea sp. Bright VIC AUSTRALIA
G. castanea MUT 401 C. sativa Robilante ITALY
G. castanea MUT 815 C. sativa Sisteron FRANCE
Gnomoniopsis smithogilvyi
99/99
1.0
76/88
-
-/81
0.83
G. paraclavulata CBS 123202
G. clavulata CBS 121255
G. idaeicola CBS 125676
G. macounii CBS 121468
G. occulta CBS 125678
79/89
0.97
G. chamaemori CBS 804.79
G. guttulata MS 0312
G. alderdunensis CBS 125680
81/97
0.99
G. racemula CBS 121469
100/99
1.0
G. tormentillae CBS 904.79
G. comari CBS 806.79
G. fructicola CBS 208.34
G. sanguisorbae CBS 858.79
Plagiostoma sp. CBS 128351
Ophiognomonia setacea CBS 128354
0.05
substitutions/site

FIG. 2. ML phylogeny of ITS sequences of *G. smithogilvyi*, *G. castanea*, and "*G. pascoe*" with single isolates of *Gnomoniopsis* reference taxa and outgroup (ML score = –lnL score of 1866.45). Country of collection, and species of *Castanea* that the isolate was collected from are next to the isolate number. Maximum likelihood bootstrap (ML), maximum parsimony bootstrap (MP) values ≥70%, and Bayesian posterior probabilities (PP) ≥0.95 are displayed at each branch in the order MLBS/MPBS/PP.

New Zealand, and Switzerland clustered in the same lineage with 99% MLBS support (FIG. 2). One isolate from France, LSV908, displayed minor interspecific variation with 1 bp different from the other isolates from *Castanea*. The MP ITS dataset had 508 total characters: 68 parsimony informative, 56 parsimony uninformative, and 384 constant. The MP heuristic search produced three most parsimonious trees with length of 228 steps (CI = 0.754386, RI = 0.87037, RCI = 0.656595 for all sites and iCI = 0.652174, iRI = 0.870370,

iRCI = 0.567633 for parsimony informative sites). The MP ITS phylogeny grouped all *Gnomoniopsis* isolates from *Castanea* in the same strongly supported clade (MPBS = 99%). The Bayesian distribution of likelihood scores for the ITS dataset was –lnL 1948.241–1998.552 and the PP of the branch supporting the isolates from *Castanea* was strongly supported with a value of 1.00.

Phylogenetic species were characterized using genealogic concordance phylogenetic species recognition (GCPSR) and the genealogical sorting incidence (GSI). The conditional comparison test (GCPSR) was satisfied for the *G. smithogilvyi* clade with MPBS for ITS = 99%, TEF1-α = 100%, β-tubulin = 100%, MS204 = 100% and FG1093 = 100%. Independent marker GSI_T values for *G. smithogilvyi* were all equal to 1 ($p \leq 0.0001–0.0005$), suggesting that this species is a distinct evolutionary lineage from other *Gnomoniopsis* species. The phylogenies of the ITS and five-marker datasets indicate all the isolates associated with *Castanea* represent one species, *G. smithogilvyi.*

Taxonomy

Gnomoniopsis smithogilvyi L.A. Shuttlew., E.C.Y. Liew & D.I. Guest, Persoonia 28: 143. 4 June 2012.

= *Gnomoniopsis castaneae* Tamietti, J. Plant Pathology 94: 416. 21 July 2012, as "*castanea.*"

Specimens examined:

Morphological and molecular examination: **AUSTRALIA**, **New South Wales**, Mullion Creek, 'Brittle Jacks' chestnut orchard, saprobic on dead burrs of *Castanea* sp., Dec. 2009, collected & isolated by L.A. Shuttleworth (holotype CBS H-20623; isotype RBG 5586; ex-type culture CBS 130190 = RBG 5585); Mullion Creek, endophytic in living leaf of *Castanea* sp., Dec 2009, collected & isolated by L.A. Shuttleworth (culture CBS 130189); **Victoria**, Bright, diseased chestnut kernel of *Castanea* sp., April 2009, collected & isolated by L.A. Shuttleworth (culture CBS 130188). **ITALY**, **Piedmont**, Robilante, diseased fruit of *Castanea sativa*, 2007, isolated by G. Tamietti & S. Gentile (culture MUT 401). **NEW ZEALAND**, diseased fruit of *Castanea sativa*, 2007, isolated by H. Smith (culture MUT 411).

Molecular examination only: **FRANCE**, **Picardy**, Oise, stem of *Castanea* sp., 2013, collected by S. Carole (culture LSV 908); **Provence-Alpes-Côte d'Azur**, Sisteron, diseased *Castanea sativa* fruit, 2011, isolated by P. Gonthier & S. Gentile (culture MUT 815). **INDIA**, bark of *Castanea sativa*, collected & isolated by M.A. Dar & M. Rai (culture AU/DBT30); bark of *Castanea sativa*, collected & isolated by M.A. Dar & M. Rai (culture AU/DBT306); on bark of *Castanea sativa*, collected & isolated by M.A. Dar & M. Rai (culture INDC(BSF5)A); bark of *Castanea sativa*, collected & isolated by M.A. Dar & M. Rai (culture AU/DBT296). **ITALY**, **Trentino-South Tyrol**, Val d'Adige, Trento, fruit with brown rot, October 2011 (culture Gn_TN5); **Piedmont**, Val di Susa, Turin, October 2011 (culture Gm_TO); **Tuscany**, Mugello, Florence, October 2011 (culture Gm_FI). **SWITZERLAND**, **Ticino**, Biasca, diseased fruit of *Castanea sativa*, 2011, isolated by M. Jermini (culture MUT 810); **Geneva**, branch wood of *Castanea sativa*, Aug 2013 (culture Ge1, specimen voucher UASWS1319). **NEW ZEALAND**, **Bay of Plenty**, Tauranga, nut of *Castanea sativa*, 1 February 2002, collected by K. Eade (culture ICMP 14492); **Waikato**, nut of *Castanea crenata*, 1 April 2000, isolated

by K.D.R. Wadia (culture ICMP 14080); from *Castanea crenata* × *C. sativa*, isolated by K.D.R. Wadia (culture CBS 121477); from *Castanea* sp., collected & isolated by H. Smith (culture CBS 121917); isolated from *Castanea crenata* (culture CBS 121918).

Ecology & distribution— *Gnomoniopsis smithogilvyi* occurs in Australia on *Castanea sativa* and hybrids of *Castanea crenata* × *C. sativa* (Shuttleworth et al. 2012a,b). In New Zealand it occurs on *C. crenata*, *C. sativa, C. crenata* × *C. sativa* hybrids, and *Castanea* sp.; and in France, India, Italy, and Switzerland on *C. sativa* (Shuttleworth et al. 2012a, Visentin et al. 2012, Maresi et al. 2013). The species is reported as a causal agent of chestnut rot/nut rot in Australia, France, Italy, New Zealand, and Switzerland (Shuttleworth et al. 2012a,b; Visentin et al. 2012), and is reported as the cause of canker of *C. sativa* in India (Dar & Rai 2013, 2015) and Switzerland (Pasche et al. 2015). The fungus is documented as an endophyte of reproductive and vegetative tissues of *C. sativa* in Australia, Italy, and New Zealand, and of *C. crenata* × *C. sativa* hybrids in Australia and New Zealand (Shuttleworth et al. 2012a,b). It also associated with necrosis of leaves and galls induced by *Dryocosmus kuriphilus* (chestnut gall wasp) on *Castanea* spp. in Italy (Magro et al. 2010; Vinale et al. 2014).

Comments— The name *Gnomoniopsis smithogilvyi* was first published on-line on 4 June 2012 in Shuttleworth et al. (2012a). The name *G. castaneae* was first published on-line on 21 July 2012 in Visentin et al. (2012) according to L. Rubino, Editor of the Journal of Plant Pathology (pers. comm.). These on-line publications were both in accordance with Article 29 of the ICN (McNeill et al. 2012). Thus, *G. smithogilvyi* has priority over *G. castaneae*. The earlier informal name, "*Gnomonia pascoe*" was never validly published.

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Distribution of *Alternaria* species among sections. 2. Section *Alternaria*

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Abstract — Among several groups of small-spored *Alternaria* species, *A.* sect. *Alternaria* (containing the generic type) has been found to be isolated, based on phylogenetic as well as morphological data. Molecular phylogenetic data confirm inclusion of twenty-one species in this section besides the type species. Examination of additional material, conforming to the morphological description for the section but not as yet phylogenetically characterized, allow confirmation of an additional 37 species. All 59 species are listed, and an emended description of *Alternaria* sect. *Alternaria* is presented.

Key words — *Alternaria alternata*, *A. herbiphorbicola*, *A. gomphrenae*, *Pseudoalternaria*

Introduction

The genus *Alternaria* Nees is a large taxonomic group that comprises approximately 280 species (Simmons 2007). A series of large-scale works has attempted to resolve the phylogeny of *Alternaria* and other alternarioid hyphomycetes by employing more than ten different genomic loci (Pryor & Bigelow 2003; Hong et al. 2005; Runa et al. 2009; Lawrence et al. 2012, 2013, 2014; Woudenberg et al. 2013). Several well-supported phylogenetic lineages have been revealed within the alternarioid hyphomycetes, resulting in several taxonomic novelties. The genus *Alternaria* was divided into eight taxonomic sections by Lawrence et al. (2013). Presenting an alternative concept, Woudenberg et al. (2013) collapsed all alternarioid hyphomycetes into one genus, *Alternaria*, with 24 sections, 11 of which referred to *Alternaria* sensu stricto.

All works support a monophyletic status of a particular *Alternaria* group, one of several groups having members with catenate, small-spored conidia.

Both research teams (Lawrence et al. 2013; Woudenberg et al. 2013) agreed that based on both molecular phylogenetic and morphological data, this group (including the type species *A. tenuis* [= *A. alternata*]) should be treated as a well-defined section. This section, which contains the type of the genus, is an autonymous section that must repeat the generic name as its epithet, *Alternaria* sect. *Alternaria*, and is not followed by any author citation (McNeill et al 2012: Art. 22.1). Lawrence et al. (2013) were mistaken in presenting this section as a sect. nov. with themselves as authors. Woudenberg et al. (2013), who repeated the erroneous attribution to Lawrence et al., misspelled the epithet as "*Alternata*".

Lawrence et al. (2013) referred thirty-one strains to *A.* sect. *Alternaria*. However, six strains belonged to *A. gossypina*, *A. grisea*, *A. grossulariae*, *A. lini*, *A. maritima*, or *A. nelumbii*, species denoted by Simmons (2007) as taxa that are not distinguishable morphologically from other small-spored *Alternaria* species, that lack isolates from identified field material, that were considered as INCERTAE SEDIS, or whose only isolate(s) sporulate inadequately. Another strain (CBS 1010.26) is labelled with the invalid name "*Alternaria iridis*", referring to *Macrosporium iridis* Cooke & Ellis, which is a *Stemphylium* (Simmons 2007: 722). Strains of three other species used by Lawrence et al. (2013) — *A. malvae*, *A. rhadina*, and *A. tomato* — were not authentic or representative. Strain CBS 595.93 was deposited as "*A. rhadina*" by the species author, E.G. Simmons, but he subsequently reported that this strain was mislabelled and did not represent an authentic *A. rhadina* (Simmons 2007: 512). Here I regard it as representing an unnamed *Alternaria* species and exclude it from my list.

An isolate of *A. tomato* (CBS 114.35) clustered in a clade referred to as *A.* sect. *Alternaria* (Lawrence et al. 2013). However, the credible species description in Simmons (2007) suggests that this species resides better in *A.* sect. *Porri*. According to Simmons (2007: 356), no correctly identified *A. tomato* isolates are known. Hence, we suggested that *A. tomato* sensu Simmons belongs in *A.* sect. *Porri* (Gannibal 2015).

Lawrence et al. (2013) placed *A. resedae* CBS 175.80 in *A.* sect. *Alternaria*. The molecular phylogenetic analyses by Woudenberg et al. (2013) placed the representative strain of *A. resedae* Neerg. CBS 115.44 in *A.* sect. *Cheiranthus*. Earlier Simmons (2007) had suggested that this name is a synonym of *A. septorioides* (Westernd.) E.G. Simmons in *A.* sect. *Brassicicola*. Obviously CBS 175.80 and CBS 115.44 belong to different species and most likely they do not both correspond to the description used by Simmons. Hence I do not recommend using the name *A. resedae* until a species study has been completed.

Based on ITS1-2 rDNA sequence analysis Hoog & Horré (2002) included 33 strains of 7 species in group *alternata*. Apart from the well-studied species *A. lini*, *A. mali* and *Cylindrocarpon lichenicola* were placed in the group. Strain "*C. lichenicola*" (current name *Fusarium lichenicola*) was obviously incorrectly identified. The status of *A. lini* has been discussed above. Strain IFO 8984 *A. mali* was not ex-type. The same strain and the type strain of *A. mali* have been studied by Rotondo et al. (2012). The strains did not demonstrate a close relationship, but both do appear to be members of *A. tenuissima*/*A. alternata* group, which is placed in *A.* sect. *Alternaria*.

Woudenberg et al. (2013) added *Alternaria daucifolii* to *A.* sect. *Alternaria,* confirming 21 species (in addition to the type species) in the section. Also three putatively representative strains corresponding to the names *Clathrospora heterospora*, *Comoclathris magna*, and *Nimbya gomphrenae* were found to be closely related to species in *A.* sect. *Alternaria*. Presumably the strains *Clathrospora heterospora* (CBS 175.52) and *Comoclathris magna* (CBS 174.52) were lost during cultivation and were replaced by *A. alternata* group isolates (Woudenberg et al. 2013). A strain of *Nimbya gomphrenae* (CBS 108.27) was isolated by Togashi and deposited at CBS in 1927, but Simmons (1989) maintained that Togashi actually isolated two fungi, one the distinctive large-spored *A. gomphrenae* [≡ *N. gomphrenae*] and the other a small-spored, chain-forming species in the *A. alternata* group. This suggests that the strain used by J. Woudenberg et al. most likely is a contaminant.

Initially *A.* sect. *Alternaria* was estimated to comprise about 60 species (Gannibal 2012; Woudenberg et al. 2013). The number of species approved by phylogenetic species recognition approaches may differ from that revealed by morphological analysis, and it is very likely that a morphological concept would favor splitting. For instance, phylogenetic analyses were performed with 150 small-spored *Alternaria* isolates using sequence data from three genomic loci (Andrew et al. 2009). Strict congruence between morphology and phylogenetic lineage was not found among isolates morphologically defined as *A. alternata* or *A. tenuissima*. Although 5–9 well-supported clades were evident among isolates, it was unclear whether these clades should be considered phylogenetic species or emerging evolutionary lineages.

In other works (Peever et al. 2004, 2005) lineages inferred from phylogenetic analysis of the combined dataset were in general agreement with described morphospecies; however, three clades contained more than one morphological species. The authors agreed to collapse all morphologically diverse small-spored, citrus-associated isolates of *Alternaria* into a single phylogenetic species, *A. alternata*.

The main diagnostic morphological feature of many *Alternaria* sect. *Alternaria* species is short conidia (usually no longer than 60 µm in culture) produced in chains (Lawrence et al. 2013; Woudenberg et al. 2013). Sexual morphs have not yet been discovered. Many strains of most well-studied species produce toxic metabolites, such as derivatives of dibenzo-α-pyrone (alternariol, alternariol monomethyl ether, altenuen, etc.), tetramic acid (tenuazonic acid), and perylene (altertoxin I, II and III) (Andersen et al. 2001, 2002; Lou et al. 2013). Almost all species in this section are saprobes or facultative parasites of plants with no strong host specialization. Only a few species (or — in an alternative classification — pathotypes of *A. alternata*) are known to be strong but specialized parasites, each able to infect only a few genotypes (cultivars) of a host plant (Tsuge et al. 2013). Such species become pathogenic due to a limited group of genes associated with synthesis of host-selective toxins, but presumably they have all the same adaptations necessary for saprotrophic growth as seen in closely related species.

It is unlikely that all 280 *Alternaria* species will be molecularly characterized in the foreseeable future. Moreover, for several species living isolates are not known and herbarium material is too old and scanty for successful DNA extraction. On the other hand, the morphological diversity of *Alternaria* is well studied and available in a monograph (Simmons 2007), and classical comparative morphological approaches can still be utilized to assign all *Alternaria* species to sections.

This work continues the series begun with *A.* sect. *Porri* (Gannibal 2015). The aim of the present work is to detect which phylogenetically unexamined *Alternaria* species fit the current morphological circumscription of *A.* sect. *Alternaria* and to emend the morphological concept of this section.

Materials & methods

Morphology of almost all *Alternaria* species with legitimate names was analyzed with regard to conformity with criteria of *Alternaria* sect. *Alternaria*. The morphological assessment was based on descriptions made by Simmons (2007). As *A.* sect. *Alternaria* is the autonymous section including the genus type, it contains all species that have not yet been segregated into other sections or designated as INCERTAE SEDIS. In this article I consider only species that are phylogenetically or morphologically closely related to the type species and do not treat "omitted" or "obscure" species.

Results & discussion

Evaluation of all available descriptions of *Alternaria* species allowed for the addition of 37 species to *A.* sect. *Alternaria,* here recognized as comprising the 59 species listed in TABLE 1.

TABLE 1. List of the 59 *Alternaria* spp. assigned to *A.* sect. *Alternaria*

A. alocasiae T.Y. Zhang & M.X. Gao [a, b]

A. alternata (Fr.) Keissl. [= *A. tenuis* Nees] (Pryor & Bigelow 2003; Hong et al. 2005; Lawrence et al. 2013; Woudenberg et al. 2013)

A. amorphophalli V.G. Rao [a]

A. angustiovoidea E.G. Simmons (Lawrence et al. 2013; Woudenberg et al. 2013)

A. arborescens E.G. Simmons (Pryor & Bigelow 2003; Hong et al. 2005; Lawrence et al. 2013; Woudenberg et al. 2013)

A. asclepiadea (Cooke) E.G. Simmons [a]

A. betae-kenyensis E.G. Simmons

A. brassicinae E.G. Simmons

A. broussonetiae T.Y. Zhang, W.Q. Chen & M.X. Gao

A. burnsii Uppal, Patel & Kamat (Lawrence et al. 2013; Woudenberg et al. 2013)

A. catharanthicola T.Y. Zhang [a, b]

A. caudata (Cooke & Ellis) E.G. Simmons

A. cerealis E.G. Simmons & C.F. Hill (Lawrence et al. 2013; Woudenberg et al. 2013)

A. citri Ellis & N. Pierce [a]

A. citriarbusti E.G. Simmons (Lawrence et al. 2013; Woudenberg et al. 2013)

A. citricancri E.G. Simmons

A. citrimacularis E.G. Simmons (Lawrence et al. 2013; Woudenberg et al. 2013)

A. colombiana E.G. Simmons (Lawrence et al. 2013; Woudenberg et al. 2013)

A. daucifolii E.G. Simmons (Woudenberg et al. 2013)

A. destruens E.G. Simmons (Pryor & Bigelow 2003; Lawrence et al. 2013; Woudenberg et al. 2013)

A. dumosa E.G. Simmons (Hong et al. 2005; Lawrence et al. 2013; Woudenberg et al. 2013)

A. floridana (Cooke) E.G. Simmons [a]

A. franseriae E.G. Simmons [a]

A. gaisen Nagano ex Hara (Lawrence et al. 2013; Woudenberg et al. 2013)

A. godetiae (Neerg.) Neerg.

A. graminum (Cooke) P. Joly [a]

A. herbiphorbicola E.G. Simmons (Lawrence et al. 2013; Woudenberg et al. 2013)

A. hesperidearum (E. Pantan.) E.G. Simmons

A. hibisci Bouhot ex E.G. Simmons [a]

A. interrupta E.G. Simmons

A. limoniasperae E.G. Simmons (Hong et al. 2005; Lawrence et al. 2013; Woudenberg et al. 2013)

A. longipes (Ellis & Everh.) E.W. Mason (Pryor & Bigelow 2003; Hong et al. 2005; Lawrence et al. 2013; Woudenberg et al. 2013)

A. mali Roberts (Rotondo et al. 2012)

A. malvae Roum. & Letendre [a]

A. obclavoidea E.G. Simmons [a]

A. palandui Ayyangar

A. pellucida E.G. Simmons
A. perangusta E.G. Simmons (Lawrence et al. 2013; Woudenberg et al. 2013)
A. petalicolor (Sorokin) E.G. Simmons [a]
A. platycodonis Z.Y. Zhang & H. Zhang
A. postmessia E.G. Simmons (Lawrence et al. 2013; Woudenberg et al. 2013)
A. pulvinifungicola E.G. Simmons
A. rhadina E.G. Simmons [a]
A. rhizophorae E.G. Simmons
A. rugosa McAlpine [a]
A. sanguisorbae M.X. Gao & T.Y. Zhang
A. senecionicola E.G. Simmons & C.F. Hill
A. soliaegyptiaca E.G. Simmons
A. spadicea (Thüm.) E.G. Simmons [a]
A. tangelonis E.G. Simmons (Lawrence et al. 2013; Woudenberg et al. 2013)
A. tenuissima (Kunze) Wiltshire (Pryor & Bigelow 2003; Hong et al. 2005; Lawrence et al. 2013; Woudenberg et al. 2013)
A. tinosporae E.G. Simmons
A. tomaticola E.G. Simmons & Chellemi
A. toxicogenica E.G. Simmons (Lawrence et al. 2013; Woudenberg et al. 2013)
A. tropaeolicola T.Y. Zhang [a, b]
A. turkisafria E.G. Simmons (Lawrence et al. 2013; Woudenberg et al. 2013)
A. uredinis (Ellis & Barthol.) E.G. Simmons [a]
A. yaliinficiens R.G. Roberts
A. zhengzhouensis T.Y. Zhang [a, b]

Names with parenthetical annotations = species with phylogenetic data (in the cited references).
Bold font names = species assigned to *Alternaria* sect. *Alternaria* previously based on phylogenetic data (Lawrence et al. 2013 and/or Woudenberg et al. 2013 or Rotondo et al. 2012).
Unannotated names = species with reliable living cultures and herbarium specimens.
Names with square bracketed annotations = species for which type material and/or representative strains are not available: [a] = no known living isolates; [b] = type specimen is not available on loan.

The most complicated task was differentiating species of *A.* sect. *Alternaria* from species belonging to *A.* sect. *Infectoria* and *Pseudoalternaria*. Typical representatives of those two groups can easily be identified by a number of cultural and microscopic morphological features (Lawrence et al. 2014). But the intermediate morphology of several species or strains tends to blur the morphological border between these two groups.

Although molecular analyses and morphological observations coincide for most species, they are discordant in the case of *A. herbiphorbicola*. In this species the conidia are short (conidial body is mainly 15–22 µm long) and produce secondary conidiophores that can slightly exceed the length

of the body. The three-dimensional sporulation pattern resulting from such relatively long secondary conidiophores with several conidiogenous loci is similar to that of *Pseudoalternaria*. But in many *Pseudoalternaria* species a portion of conidia usually have longer apical secondary conidiophores (30–80 µm). In *Pseudoalternaria* lateral secondary conidiophores usually have 1–2 conidiogenous loci, while several species of *Alternaria* sect. *Alternaria*, including *A. herbiphorbicola*, can produce such conidiophores with up to 4 pores.

Despite ambiguities resulting from the lack of authentic living cultures, both *A. malvae* and *A. rhadina* should be placed in *A.* sect. *Alternaria* based on the morphological characters documented by Simmons (2007).

Here I emend (and somewhat expand) the description of *Alternaria* sect. *Alternaria* given previously by Lawrence et al. (2013). Characters are quantified when appropriate, and examples are provided for species whose quantified characters either depart from the section norm or conform to it.

Alternaria **Nees sect. *Alternaria*** emend. Gannibal

Type: *Alternaria tenuis* Nees [= *Alternaria alternata*]

On PCA primary conidiophores are straight or curved, simple or branched, short (15–60 µm; *A. brassicinae*, *A. gaisen*) or long (≤300–435+ µm; *A. betae-kenyensis*), 3-4 µm wide. Conidiophores bear one or several (ranging from 1–2 to 1–5 in different species) conidiogenous loci at the apex. Conidia form simple or branched chains that are moderately long (2–6 conidia; *A. godetiae*, *A. toxicogenica*) to long (10–20+ conidia; *A. daucifolii*, *A. perangusta*). Conidial aggregates usually present as simple straight to branched chains of conidia or as loose to tightly packed branching clusters (≤125 conidia; *A. alternata*). Young terminal conidia often are short ovoid or ellipsoid. Mature conidia are obclavate, long ellipsoid or ellipsoid, septate, 15–50(–60) × 7–13 µm in culture and somewhat larger in nature (long secondary conidiophores are not included in calculation). Conidia mainly distoseptate, with numerous transverse septa (ranging from 3–7 to 5–12 in different species) and 1–2 longitudinal septa in one or a few of the transverse divisions. In some species fully developed conidia have 1–3 eusepta. Conidia are slightly constricted near eusepta and sometimes near some distosepta. Most conidia narrow gradually into a tapered beak or secondary conidiophore. Apical secondary conidiophores are short, usually not exceeding 5–10 µm (but sometimes longer, in some species ≤100–110+ µm; *A. angustiovoidea*, *A. tinosporae*), bearing one to several conidiogenous loci (ranging from 1–2 to 1–4 in different species). Some conidia produce short solitary lateral secondary conidiophores. They are simple or with very short branches, 5–10(20)×3–4 µm, with 1-4 conidiogenous loci.

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The lichen genus *Kroswia* in China

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Abstract —Three species belonging to the lichen genus *Kroswia* are reported from China: *Kroswia epispora* sp. nov., *K. crystallifera*, and *K. gemmascens*. The colourless ellipsoid ascospores with epispore in *K. epispora* provide new evidence for the close relationship between *Kroswia* and *Fuscopannaria*. Descriptions and comments for the three species and a key to all *Kroswia* species are presented.

Key words —*Ascomycota*, *Pannariaceae, Peltigerales,* taxonomy

Introduction

Kroswia P.M. Jørg. is a small, paleotropical genus belonging to *Pannariaceae*, *Peltigerales*, *Lecanoromycetidae*, *Ascomycota.* It differs from other genera in the family by its gelatinous, homoiomerous, and ecorticate thallus and the presence of terpenoids and fatty acids (Jørgensen 2002, 2003a). The genus contains three species: *Kroswia crystallifera* (the type), *K. gemmascens*, and *K. polydactyla* (Jørgensen 2002, 2003a, 2004; Jørgensen & Gjerde 2012).

Only *K. gemmascens* is known to be fertile, and its hymenium characters suggest a close relationship with *Fuscopannaria* P.M. Jørg. (Jørgensen 2007). This was further confirmed by a recent molecular study suggesting that *K. cystallifera*, the type of the genus, should be included in *Fuscopannaria* (Magain & Sérusiaux 2014), making the genus *Kroswia* a later synonym of *Fuscopannaria* (Magain & Sérusiaux 2015). However, another recent molecular phylogeny of *Pannariaceae* failed to obtain PCR products from *Kroswia* samples, and the authors therefore retained *Kroswia* as a separate genus (Ekman et al. 2014). Because the placement of the other two *Kroswia* species in *Fuscopannaria* lacks molecular support, we follow here the traditional concept of *Kroswia*.

Two *Kroswia* species have been reported from China, *K. crystallifera* from Taiwan (Jørgensen 2002) and *K. gemmascens* from mainland China (Zahlbruckner 1930; Wei 1991; Jørgensen 2002, 2007). Here we describe *Kroswia epispora*, and present a key to all *Kroswia* species.

Materials & methods

Specimens are deposited in Herbarium Mycologicum Academiae Sinicae-Lichenes, Beijing, China (HMAS-L), the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China (KUN), the Herbarium of Nanjing Normal University, Nanjing, China (NJNU), and the Herbarium of Shandong Normal University, Jinan, China (SDNU). Dissecting microscopes (Motic SMZ-140 and Olympus SZ51) and light microscopes (Motic B2 and Olympus CX21) were used for the morphological and anatomical studies. Photographs were taken with BX51 fluorescence microscope.

Color tests and thin-layer chromatography (TLC) were routinely performed as described by Culberson & Kristinsson (1970), Culberson (1972), Culberson et al. (1977), Huneck & Yoshimura (1996), and Orange (2010). The K, C, and P reagents were used for color tests. The solvent system C for TLC was used to develop acetone extraction of the thallus fragments on a silica gel-coated glass plates. Lugol's solution was added to the apothecial sections for Iodine reaction.

Taxonomy

Kroswia epispora H.J. Liu & Chao Li, **sp. nov.** Pl. 1

MycoBank MB 811551

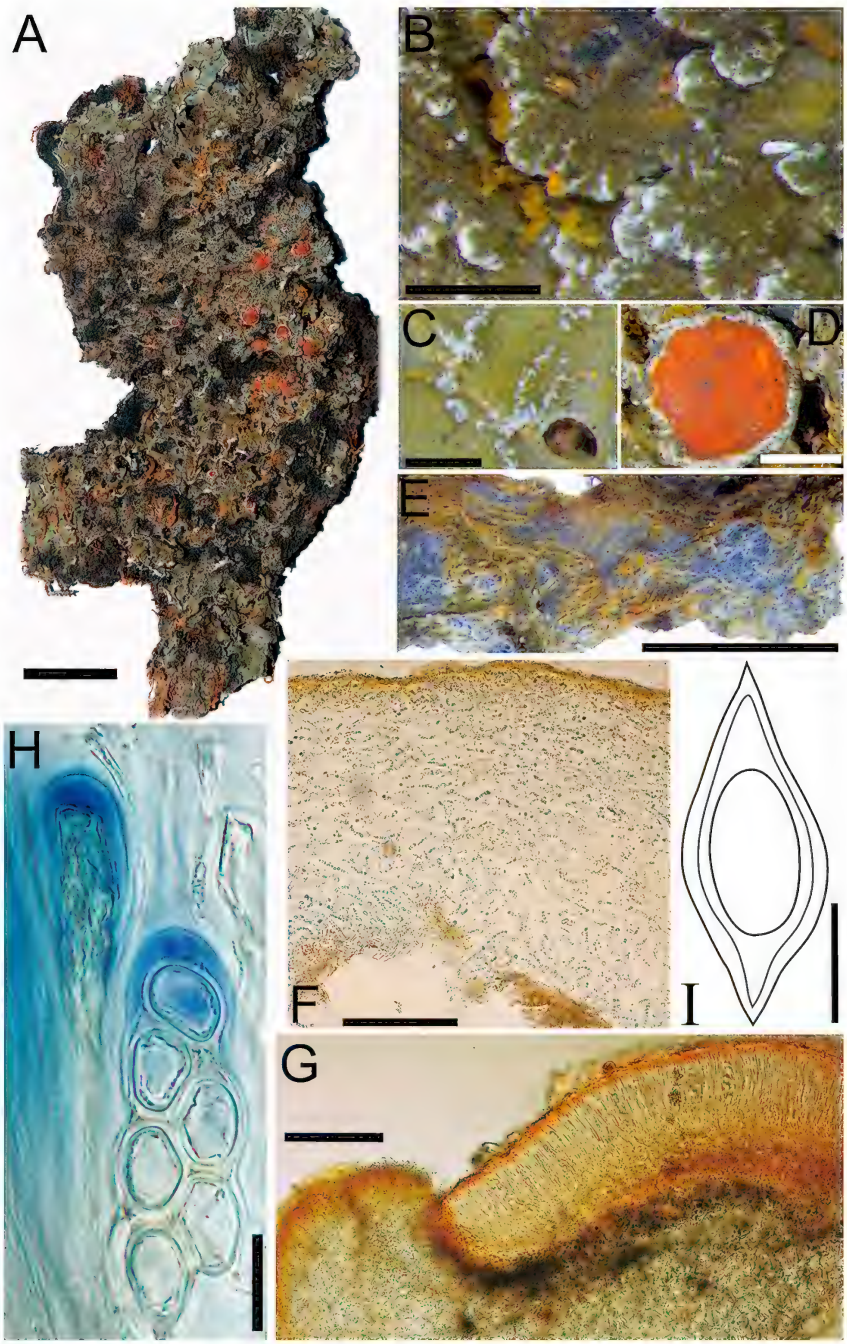
Differs from *Kroswia crystallifera* by the presence of apothecia and absence of gymnidia.

Type: China. Zhejiang Province, Mt. Jiulongshan, 28°22 N 118°52 E, alt. 1600 m, on bark, 8/V/1987, Shu-Fan Chen Z0923 (**Holotype**, NJNU).

Etymology: The epithet refers to the epispore of the ascospores.

Thallus foliose, orbicular to irregular spreading, 4–9 cm in diam., partially fenestrate, without distinct hypothallus; lobus rounded, up to 4–5 mm broad; upper surface olivaceous brown, glabrous, uneven to irregularly warted, sparsely wrinkled (warts and part of wrinkles sometimes bearing white powder crystals), without gymnidia, marginally with white marbling and bearing white terpenoid crystals; lower surface bluish gray near margin, blackened towards center, smooth to finely wrinkled, with scattered tufts of rhizines. Apothecia

Plate 1. ***Kroswia epispora*** (holotype, NJNU Chen, Z0923). A. Thallus with apothecia; B. Upper surface, showing lobe margins with white terpenoid crystals; C. Upper surface, showing fenestrated thallus and sparse wrinkles with white powder crystals; D. An apothecium, showing thalline margin with white powder crystals; E. Lower surface; F. Cross-section of thallus; G. Cross-section of apothecium, showing the ecorticate and well-developed apothecial margin; H. Ascus and ascospores, showing the amyloid ring structure of ascus apex, and subglobose ascospores in ascus; I. Ascospores, showing distinct, smooth epispore. Scale bars: A= 1 cm; B, C = 0.4 mm; D = 1 mm; E = 5 mm; F, G = 150 µm; H = 20 µm; I = 10 µm.


A
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I

common, laminal, mainly adnate upon the center of the thallus, 2–3 mm in diam.; DISC plane to convex, reddish brown; THALLINE EXCIPLE thick, glabrous, smooth to warted, crenate at margins, beset with white powder crystals.

THALLUS homoiomerous, 300–400 μm thick, ≤500 μm thick when wet; BOTH CORTICES lacking, brown at the uppermost 20 μm and lowermost 10 μm portions, colourless or somewhat bluish at the rest portions; PHOTOBIONT *Nostoc* in long chains, consisting of more than 10 cells of 4–5 μm in diam.; HYPHAE regularly interwoven.

APOTHECIA lecanorine; THALLINE EXCIPLE well-developed, 350–450 μm thick at the margin, without cortex, with an algal layer of long chains of *Nostoc* and radiating hyphae; PROPER EXCIPLE poorly-developed or lacking; HYMENIUM 100–140 μm high, hemiamyloid (I+ blue, rapidly turning red-brown), with a thin, pale yellow-brown epithecium; PARAPHYSES simple, 1.5–3 μm thick, slightly thickened at the apices; SUBHYMENIUM well-developed, ≤100 μm high, of non-oriented, closely compacted hyphae; ASCI cylindrical-clavate, apically with an internal amyloid ring structure, 8-spored; ASCOSPORES colourless, subglobose to broadly ellipsoid in asci, ellipsoid when released, tapered at both ends, with visible, smooth epispore, 25–32 × 10–12 μm.

Corticolous.

CHEMISTRY: All reactions negative (color test); terpenoids and fatty acids present (TLC).

ADDITIONAL SPECIMENS EXAMINED: **CHINA. SICHUAN PROVINCE**, Mt. Emeishan, on bark, alt. 3160 m, 17/VIII/1963, J.D. Zhao & L.W. Xu 07659 (HMAS-L 036844), 7773-1 (HMAS-L 131145); alt. 3100 m, on bark, 14/XI/1964, J.C. Wei 2252-1 (HMAS-L 131144), 2903-1 (HMAS-L 105161).

REMARKS: The new species is corticolous in montane forests from Sichuan and Zhejiang Provinces of China. It is characterized by a homoiomerous thallus without cortices, white marbled lobe margins, ellipsoid colourless ascospores with an epispore, absence of gymnidia, and presence of terpenoids and fatty acids.

Kroswia gemmascens is also fertile, but has gymnidia and light brown ascospores without epispore; its globose, brown-pigmented ascospores are unique in *Pannariaceae* and an important character distinguishing *Kroswia* from *Fuscopannaria* (Jørgensen 2007, Ekman et al. 2014). The ellipsoid colourless ascospores in *K. epispora* are similar to those in *Fuscopannaria*, providing new evidence for the close relationship between the two genera.

Kroswia polydactyla P.M. Jørg. also lacks gymnidia on the thallus, but it has dactyliform lobes and has no lichen substances detectable by TLC. The new species may be a fertile counterpart of *K. crystallifera*. Both species are morphologically very similar, but *K. crystallifera* is sterile with abundant gymnidia.

Kroswia crystallifera P.M. Jørg., Lichenologist 34(4): 299 (2002). Pl. 2 A–C

≡ *Fuscopannaria crystallifera* (P.M. Jørg.) Magain & Sérus., Lichenologist 47(1): 39 (2015).

Thallus foliose, membranaceous, irregular spreading, 3–9 cm in diam., partly fenestrate, without distinct hypothallus; lobus flattened, flabellate, up to 3 mm broad; upper surface olivaceous grey to olivaceous brown, glabrous, sparsely wrinkled, with sparse to dense, white marbling, marginally beset with bluish grey gymnidia (sometimes also spreading to upper surface); gymnidia always covered with white terpenoid crystals; lower surface bluish gray near margin, blackened towards center, distinctly veined, with scattered tufts of blackish rhizines.

Thallus homoiomerous, ≤500 μm thick when wet, brown near both sides, the rest portions colourless; both cortices lacking; photobiont *Nostoc* in chains, small-celled, 4–6 um in diam.; hyphae loosely interwoven.

Apothecia not seen.

On bark, rarely on moss covered rocks.

Chemistry: All reactions negative (color test); terpenoids present (TLC).

Specimens examined: **China. Anhui Province**, Mt. Huangshan, on bark, alt. 1330 m, 09/VIII/1978, Z.G. Qian, 01484a (NJNU); alt. 1000 m, 09/IX/1981, Z.G. Qian 03203 (NJNU). **Guizhou Province**, Mt. Fanjingshan, on shrubs, alt. 2100 m, 04–05/VII/1988, L.S. Wang 88-187, 88-192, 88-248 (KUN). **Hubei Province**, Shennongjia, on bark, alt. 2650 m, 20–30/VII/1984, A.T. Liu 8401312, 8401430, 8401510 (NJNU); alt. 1350 m, 09–10/VIII/1984, J.N. Wu 8401821, 8401867 (NJNU). **Sichuan Province**, Yanyuan County, on bark, alt. 3450 m, 26/VII/1983, L.S. Wang 83-1339 (KUN); alt. 3100 m, 11/VIII/1983, L.S. Wang 83-1509 (KUN); Luding County, Mt. Gonggashan, on rotten branches, alt. 2800 m, 01/IX/1996, L.S. Wang 96-16287 (KUN). **Zhejiang Province**, Mt. Jiulongshan, on bark, alt. 980–1450 m, 4–8/V/1987, S.F. Chen Z1334, Z1348, Z0544 (NJNU); 29/IV/1987, S.F. Chen Z0792, Z0796 (NJNU); Mt. Tiantaishan, on bark, alt. 730 m, 24/VIII/1986, Z.G. Qian, 04514 (NJNU).

Remarks: The species is characterized by a glabrous sparsely wrinkled and white marbled upper surface, lobe margins with gymnidia, a veined lower surface, a homoiomerous thallus without cortices, and the presence of terpenoids. It has been reported from Australia, East Africa, Madagascar, Reunion, South Asia, and Taiwan of China (Jørgensen 2002, 2003a, b, 2004; Magain & Sérusiaux 2014). The new materials reported here were collected on barks in subtropical montane forests and extend the Chinese range from Taiwan westward to southern Sichuan.

Kroswia crystallifera is the type of the genus (Jørgensen 2002), and has been suggested to belong to *Fuscopannaria* (Magain & Sérusiaux 2014, 2015), from which it differs, however, by the homoiomerous thallus without proper cortices. It differs from *K. gemmascens* in the white marbled upper surface (especially noticeable near the margins), less wrinkled lobes, a fenestrate foliose thallus,

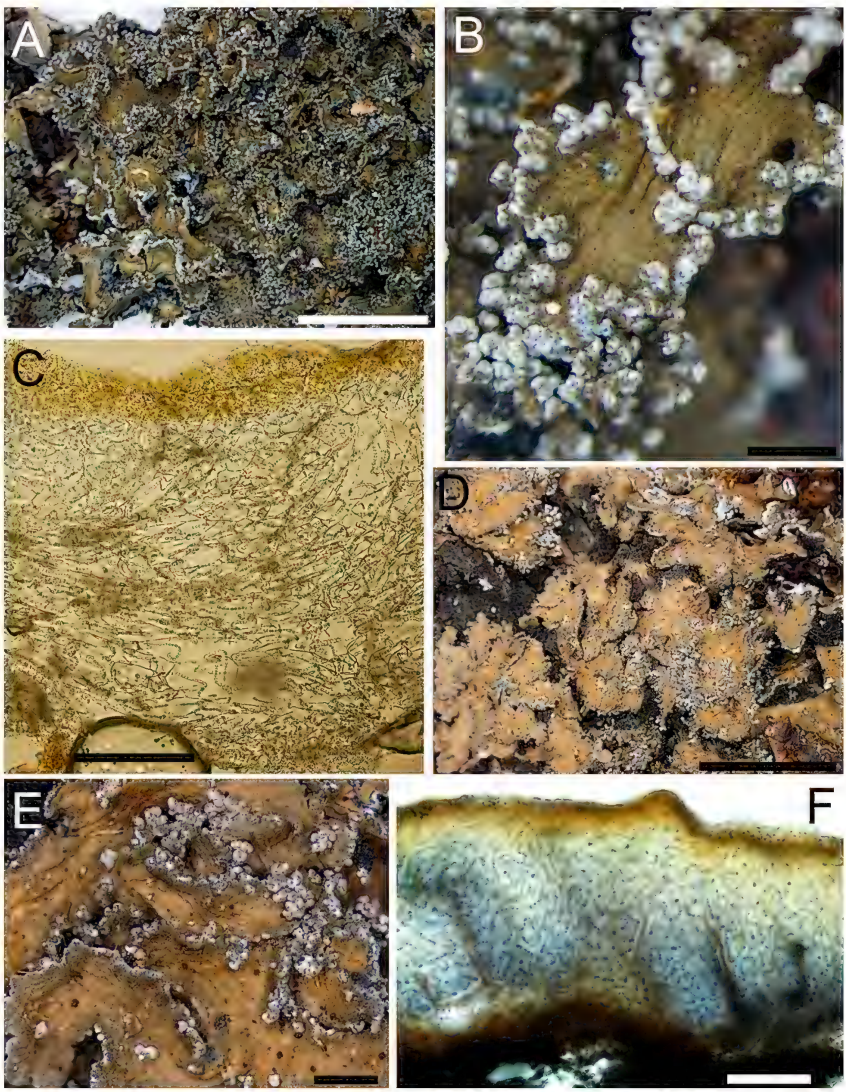

Plate 2. ***Kroswia crystallifera*** (NJNU Liu 8401430). A. Thallus; B. Lobes, showing slightly wrinkled upper surface and gymnidiate lobe margins; C. Cross-section of thallus. ***Kroswia gemmascens*** (HMAS-L 073208). D. Thallus; E. Lobes, showing wrinkled upper surface and gymnidiate margins; F. Cross-section of thallus. Scale bars: A, D = 1 cm; B, E= 0.5 mm; C, F = 100 μm.

and the absence of apothecia. *Kroswia epispora* and *K. polydactyla* differ by the absence of gymnidia.

Wu & Qian (1989) reported *Physma gemmascens* and *P. pergranulatum* (both synonyms of *K. gemmascens*) from eastern China without citing any specimens, but most of the specimens in NJNU that they identified by these names represent *K. crystallifera*.

Kroswia gemmascens (Nyl.) P.M. Jørg., Lichenologist 34(4): 302 (2002). Pl. 2D–F

≡ *Pannaria gemmascens* Nyl., Lich. Japon.: 36 (1890).

≡ *Physma gemmascens* (Nyl.) Asahina, Journ. Jap. Bot. 38: 66 (1963).

= *Physma pergranulatum* Zahlbr., Symb. Sin. 3: 75 (1930).

Thallus squamulose, ≤1.5 cm diam., forming rounded to irregular rosettes ≤3–7 cm diam., without distinct hypothallus; lobes rounded to somewhat elongated, auriform, 1–2 mm wide; upper surface light brown to dark brown, without white marbling, often wrinkled when dry, gelatinous and considerably swelling when wet; gymnidia marginal, rarely also spreading to upper surface, granular, bluish-white, often covered with white terpenoid crystals; lower surface marginally light brown to whitish, darker towards the center, partly with bluish-black rhizohyphae in the center.

Thallus homoiomerous, ≤400 µm when wet; both cortices lacking; photobiont *Nostoc* in chains.

Apothecia not seen.

On barks and mosses.

Chemistry: All reactions negative (color test); terpenoids and fatty acids present (TLC).

Specimens examined: **CHINA. Anhui Province**, Mt. Huangshan, on bark, alt. 1610 m, 20/VIII/1962, J.D. Zhao & L.W. Xu 5532 (HMAS-L 073208). **Guizhou Province**, Kaili City, Mt. Leigongshan, on bark, alt. 1800 m, 23/VIII/2010, D.F. Jiang 20102857 (SDNU); Daozhen County, Dashahe, on bark, alt. 1500 m, 10/VI/1987, X.L. Wu 2960 (HMAS-L 073223). **Hunan Province**, Guidong County, Sidu Town, on mosses, 17/VIII/2000, M.R. Huang 005 (HMAS-L 036431). **Jiangxi Province**, Mt. Lushan, on mosses, alt. 1150 m, 11/II/1965, J.C. Wei 3129 (HMAS-L 036429).

Remarks: The species is characterized by a squamulose non-fenestrate thallus, auriform lobes, a wrinkled and non-marbled upper surface, marginal gymnidia, and a homoiomerous non-corticate thallus. It is a fertile species with globose brown spores (Jørgensen 2007), but our materials are all sterile.

The species has been recorded from Japan and China [Sichuan, Xizang, and the Jiangxi/Fujian border] (Jørgensen 2002, 2003a, 2007; Zahlbruckner 1930, as *Physma pergranulatum*). The new materials reported here were collected on barks or mosses from montane forests and extend the Chinese range to Anhui, Guizhou, Hunan, and Jiangxi Provinces.

Wu & Qian (1989) reported this species (without citing any specimens) from Anhui, Fujian, and Jiangxi (as *Physma pergranulatum*) and from Zhejiang

and Hubei (as *P. gemmascens*), but most of the specimens at NJNU that they identified by these names represent *Kroswia crystallifera*. Both *K. gemmascens* and *K. crystallifera* are gymnidiate species; for a comparison see the remarks under *K. crystallifera*, above.

Key to species of *Kroswia*

1 Gymnidia present 2
Gymnidia absent 3

2 Thallus foliose; lobes flabellate; upper surface sparsely wrinkled; apothecia and ascospores unknown ..... *K. crystallifera*
Thallus squamulose; lobes auriform; upper surface densely wrinkled; ascospores globose, pale brown, without a distinct epispore ..... *K. gemmascens*

3 Lobes dactyliform; terpenoids absent; apothecia unknown ..... *K. polydactyla*
Lobes flabellate; terpenoids present; ascospores ellipsoid, colourless, with a distinct epispore ..... *K. epispora*

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Volume 130, pp. 961–965 *October–December 2015*

New reports of macromycetes from Mexico

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Abstract — Two species of macromycetes, *Phellodon tomentosus* and *Pulvinula constellatio,* are reported by first time for the Mexican mycobiota. The studied materials were collected from the state of Jalisco in pine-oak forest.

Key words — *Ascomycota*, *Basidiomycota*, taxonomy

Introduction

The present work contributes records of two genera poorly known from Mexico. *Pulvinula* Boud. *(Pyronemataceae, Ascomycota)* has not previously been reported from the Mexican mycobiota (Medel & Guzman 1999, Medel 2007), and only four species of *Phellodon* P. Karst. (*Bankeraceae, Basidiomycota*) have been reported from Mexico: *Phellodon alboniger* (Peck) Banker, *P. confluens* (Pers.) Pouzar, *P. melaleucus* (Sw. ex-Fr.) P. Karst., and *P. niger* (Fr.) P. Karst. (Cifuentes 1999).

Materials & methods

Specimens were collected from a pine–oak forest in the Mexican state of Jalisco, and deposited at the herbarium of the Institute of Botany of the University of Guadalajara, Mexico (IBUG). Macro- and micro-morphological descriptions and drawings were made of the studied materials. Photographs of fruiting bodies are provided for each taxon. Microscopic sections were mounted in a 3% KOH aqueous solution and Melzer's reagent. Specimens were identified after consulting Cifuentes (1999), Dennis (1970, 1978), Maas-Geesteranus (1975), Hall & Stuntz (1971), and Stalpers (1993).

Species descriptions

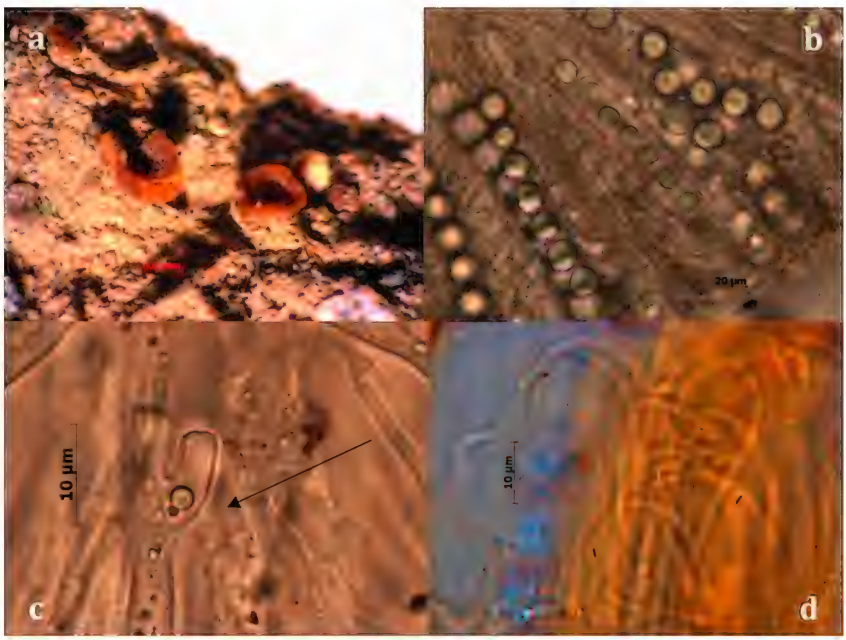

Plate 1. *Pulvinula constellatio* (Rodríguez 2359): a) discoid ascomata, b) asci with globose ascospores, c) ascal crozier, d) septate paraphyses with curved hyaline tips.

Pulvinula constellatio (Berk. & Broome) Boud., Hist. Classific. Discomyc. Europe: 70. 1907. Plate 1

Apothecia 1–5 mm diam., discoid, reddish orange and lighter orange pink towards the center, surface glabrous, margin finely wavy. Ascospores 13–15(–16) µm, globose uniseriate in the ascus, hyaline with numerous drops. Asci 216–246 × 13–14 µm, cylindrical with bifurcate base formed by a crozier, hyaline. Paraphyses 2 µm diam., filiform, slender with curved apex, forked, septate with numerous yellowish drops.

Specimen studied — **MEXICO. Jalisco**: Municipality of Zapopan, El Valle de las Tortugas, La Primavera, 17 Oct 2000, O. Rodríguez 2359 (IBUG).

Habitat — On ground, gregarious, in pine-oak.

Comments — Our material matches the description by Breitenbach & Kränzlin (1984) in ascospore morphology (13–17 µm diam., globose, with one or several drops) and the bifurcating paraphyses with curved apices. Dennis (1978) also described 13–15 µm diam. ascospores and curved branched paraphyses. Our specimen, however, had septate paraphyses whereas no septa were noted by those authors.

According to Dennis (1978), the distinctive forked ascal base of *Pulvinula constellatio* is also found in *P. cinnabarina* (Fuckel) Boud., which differs by its larger ascospores (≤18 µm diam.) and asci (≤320 µm long). Lantieri (2008) described *P. cinnabarina* with ascospores of 15-16.5 µm diam. and asci of 250 × 17-18 µm.

Pulvinula differs from *Lamprospora* by its more pulvinate ascoma and its slender and much forked non-clavate paraphyses with curved apices (Dennis 1978).

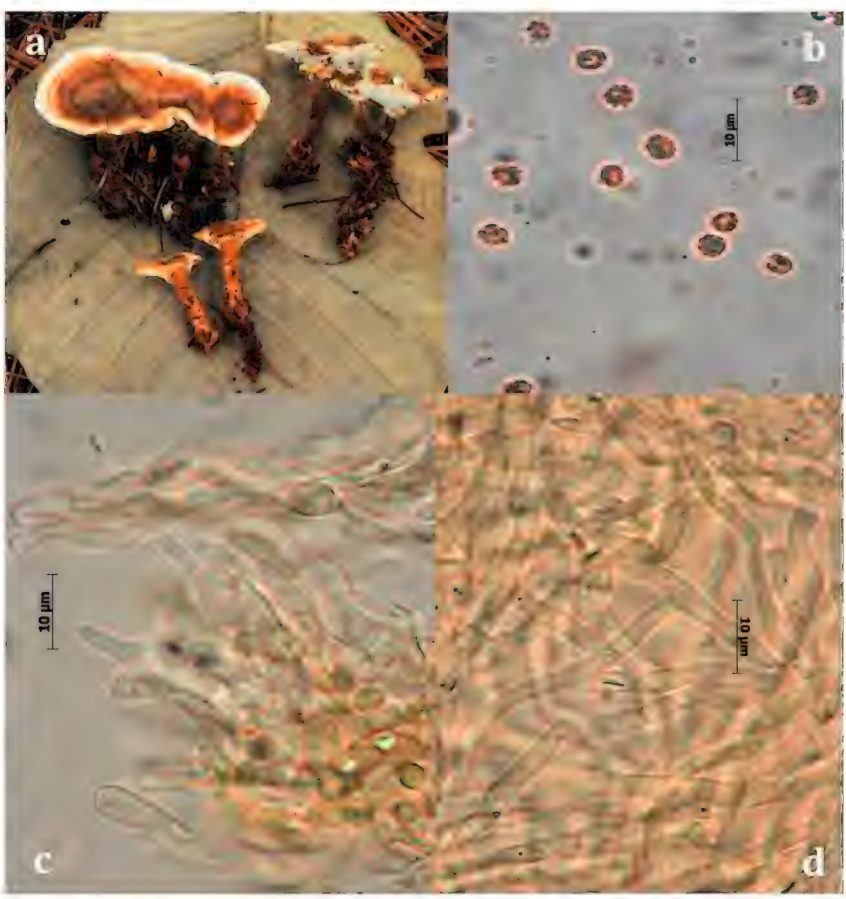

Plate 2. *Phellodon tomentosus* (Herrera 1260): a) basidiomes, b) subglobose echinulate (spiny) basidiospores, c) lageniform hyaline cheilocystidia, d) generative hyphae.

Phellodon tomentosus (L.) Banker, Mem. Torrey Bot. Club 12: 171. 1906. Plate 2

Pileus 20–35 mm diam., zonate, velutine to tomentose, more conspicuous towards the center, with the margin white to whitish-yellowish and the disc

orange-brown to reddish-brown and dark brown. Hymenophore toothed or with spines, whitish to cream coloured, with gray-pink tones in dried material. Stipe central to eccentric, surface also zonate and concolorous with the pileus but obscured in the base by a reddish-brown colour. Context zonate, reddish-brown, woody, odor agreeable, nut-like.

Basidiospores 3–4.5 × 3-4 µm, subglobose, echinulate, hyaline. Cheilocystidia 29–37 × 4–6 µm, lageniform, thin-walled, the majority with a long neck and some flexuose, hyaline, abundant, with basal septa and clamp connections absent. Hyphal system monomitic with generative hyphae 1.5–4 µm diameter, light yellowish coffee colour, and without clamp connections.

Specimen studied — **MEXICO. Jalisco**: Municipality of San Sebastián del Oeste, Camino Real Alto – La Bufa, 5.5 km del pueblo de San Sebastián del Oeste, 1 Aug 2009, M. Herrera 1260 (IBUG).

Habitat — On soil and needle litter, gregarious, in pine-oak.

Comments — *Phellodon tomentosus* is characterized by circular stipitate basidiomes that form into small colonies of five or six specimens with fused pilei, each pileus having its own independent stipe. Although cheilocystidia have not previously been reported for *Phellodon* species (Maas-Geesteranus 1975, Pegler et. al. 1997, Stalpers 1993), we observed cheilocystidia over the entire hymenial surfaces of our material.

The macro- and micro-morphological features agree with those described by Breitenbach & Kränzlin (1986). *Phellodon tomentosus* can be confused with some *Hydnellum* species based on context and stipe zonation, but *Hydnellum* can be differentiated microscopically by its brown spores.

Phellodon tomentosus is a widely distributed but uncommon species; it is reported as mycorrhizal on conifers (Pegler et al. 1997) and in mixed forests (Maas-Geesteranus 1975).

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Two new records of microfungi from the Brazilian Atlantic Forest

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Abstract— Two microfungi, *Linodochium sideroxyli* and *Leptodiscella africana*, are recorded for the first time from Brazil. The specimens, collected on decaying leaves in natural areas of the Brazilian Atlantic Forest, are described and illustrated.

Key words— taxonomy, conidial fungi, tropics

Introduction

Linodochium Höhn. (Höhnel 1909, Dyko & Sutton 1979, Li et al. 2013), typified by *L. hyalinum*, comprises five species characterized by cupulate, superficial, separate or confluent, and yellow, orange, or white sporodochial conidiomata, closely packed branched conidiophores, and polyblastic, denticulate, sympodially extended conidiogenous cells. The conidia are solitary, cylindrical or falcate, septate, and hyaline (Castañeda-Ruiz & Kendrick 1990a, Li et al. 2013).

The genus *Leptodiscella*, introduced by Papendorf (1969) and typified by *L. africana*, is distinguished by mono- or polyblastic, denticulate, sympodially extended conidiogenous cells and solitary, cylindrical to oblong, 1-septate, hyaline conidia with a subpolar, filiform appendage at each cell. *Leptodiscella* comprises four species (Madrid et al. 2012).

During three mycological surveys of conidial fungi occurring on leaf litter from the Atlantic rainforest, the microfungi *Linodochium sideroxyli* and *Leptodiscella africana* were found and recorded for the first time in Brazil.

Material & methods

Samples of leaf litter were placed in paper and plastic bags, taken to the laboratory, and treated according to Castañeda-Ruiz (2005). Mounts were prepared in PVL (polyvinyl alcohol and lactic acid) and measurements were taken at ×1000. Micrographs were obtained with a Nikon microscope (model Eclipse Ni-U) with bright field and DIC optics. Specimens were deposited in the Herbarium of Universidade Federal de Pernambuco, Recife, Brazil (URM).

Taxonomy

Linodochium sideroxyli Dulym., P.M. Kirk & Peerally,
Mycotaxon 73: 317. (1999). Fig. 1A–D

Colonies on the natural substrate sporodochial, cupulate or pulvinate, scattered, amphigenous, yellow, 300–550 µm diam. Mycelium superficial and immersed, composed of septate, branched, smooth, hyaline hyphae, 1–3 µm diam. Conidiophores distinct, branched, erect or flexuous, strongly geniculate above, numerous, tightly aggregated, 1–3-septate, smooth, hyaline, 25–50 × 2–4 µm. Conidiogenous cells polyblastic, discrete, terminal, indeterminate, long sympodial elongated, with broad, flat-topped denticles forming a geniculate rachis, hyaline, 15–30 × 2–4 µm. Conidial secession schizolytic. Conidia solitary, cylindrical-filiform, slightly inequilateral, basal cell 1 µm wide, apical cell 1.5–2 µm diam., hyaline, smooth, 0–1-septate, mostly 1-septate, with very thick and refractive walls at each end, truncated at the base, rounded at the apex, 33–40 × 1.5–2 µm

specimens examined: **BRAZIL. Pernambuco: Cabo de Santo Agostinho,** Refúgio de Vida Silvestre Matas do Sistema Gurjaú, 8°13′S 35°03′W, on decaying leaf of an unidentified plant, 12-XI-2014, coll. M.A. Barbosa (URM 87703); **Abreu e Lima,** Mata de Monjope, 7°52′S 34°55′W, on decaying leaf of an unidentified plant, 30-III-2015, coll. M.A.G. Araújo (URM 87704).

Note: The Brazilian specimens have shorter conidia than those of the type material (32–48 µm long; Dulymamode at al. 1999) but otherwise fit well the original description.

Leptodiscella africana (Papendorf) Papendorf,
Trans. Br. Mycol. Soc. 53: 146 (1969). Fig. 1E–H

Conidiomata on the natural substrate sporodochial, scattered or confluent, white. Mycelium superficial and immersed, compose of septate, branched, subhyaline hyphae, 1–1.5 µm diam. Conidiophores distinct, erect or prostrate, subhyaline, 0–2-septate, ≤25 µm long, sometimes reduced to conidiogenous

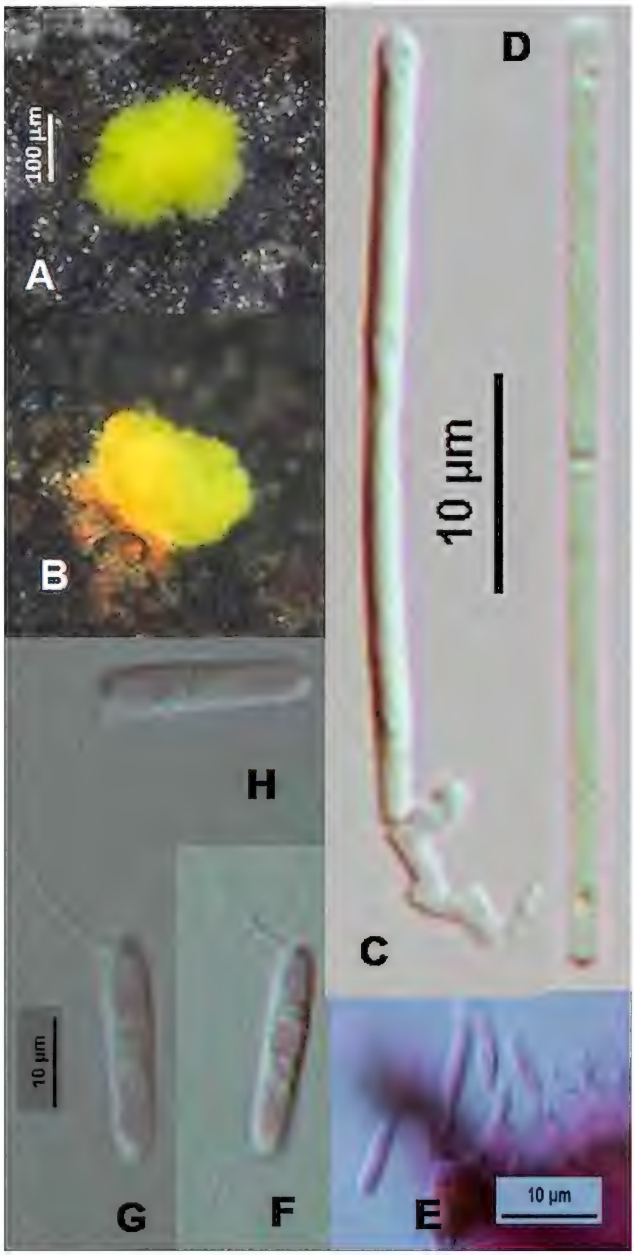

Fig 1. ***Linodochium sideroxyli*** (URM 87704): A, B. Sporodochia on the natural substratum. C. Conidiogenous cell and conidium. D. Conidium ***Leptodiscella africana*** (URM 86557): E. Conidiogenous cells and conidia. F–H. Conidia.

cells. Conidiogenous cells mono- and polyblastic, terminal and intercalary, sympodial extended, cylindrical or irregular, subhyaline, smooth, 5–10 × 1–2 µm. Conidial secession schizolytic. Conidia solitary, cylindrical or oblong, 1-septate, rounded or slightly obtuse at the ends, sometime somewhat truncate at the base, hyaline smooth, 12–18 × 2–3 µm, with a filiform, subpolar appendage at each end, 6–8 µm long.

specimen examined: **BRAZIL. Pernambuco: Cabo de Santo Agostinho,** Refúgio de Vida Silvestre Matas do Sistema Gurjaú, 8°13′S 35°03′W, on decaying leaf of an unidentified plant, 12-XI-2014, coll. M.A. Barbosa (URM 86557).

Note: The original description of *Leptodiscella africana* was derived from a pure culture on potato-carrot-agar (Papendorf 1967, 1969); however, the conidial sizes in the type material (11–17.5 × 2–3 µm) are almost the same as in the Brazilian specimen, although the subpolar conidial appendages from the type are slightly longer than from the Brazilian specimen (6.5–13 µm).

Acknowledgments

The authors express their sincere gratitude to Dr. Josiane S. Monteiro and Dr. De-Wei Li for their critical review of the manuscript. The authors are grateful to the 'Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)' for financial support through project 88881.062172/2014-01 and the 'Programa Ciência sem Fronteiras' RFCR is grateful to the Cuban Ministry of Agriculture and 'Programa de Salud Animal y Vegeta', project P131LH003033 for facilities. Dr. Lorelei Norvell's editorial review and Dr. Shaun Pennycook's nomenclature review are greatly appreciated.

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Pyramidospora quadricellularis sp. nov. on submerged leaves from Brazil

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Abstract — *Pyramidospora quadricellularis*, a new species collected on submerged decaying leaves in Brazil, is described and illustrated. It is characterized by 4-celled, hyaline, smooth conidia. Illustrations and a comparative table of the described species of *Pyramidospora* are provided.

Key words — asexual fungi, systematics, freshwater fungi

Introduction

Pyramidospora, established by Nilsson (1962) and typified by *P. casuarinae* Sv. Nilsson, comprises eight species (Ingold 1975, Index Fungorum 2015). The genus is distinguished by conidiophores that are distinct, septate, and hyaline and monoblastic determinate terminal conidiogenous cells. The conidia are more or less pyramid-like, stellate to stauroform, and multicellular, composed of hyaline globose cells that sometimes form a moniliform branched or irregularly complex conidial body. A fungus collected during a mycological survey of freshwater fungi associated with submerged leaves in streams at a Brazilian Atlantic Forest is herein described as new *Pyramidospora* species and compared with other described species (Table 1).

Materials & methods

During an expedition in April 2015 through "Refúgio de Vida Silvestre Matas do Sistema Gurjaú," samples of submerged decaying leaves were placed in sterile glass jars

with water from the site, taken to the laboratory, and processed according to Ingold (1975) and Schoenlein-Crusius & Milanez (1989). Mounts were prepared in lactic acid 90%, and measurements made at a magnification of ×1000. Photomicrographs were obtained with a Nikon Eclipse Ni-U microscope with bright field and DIC optics. The holotype was deposited in the Herbarium of Universidade Federal de Pernambuco, Recife, Brazil (URM).

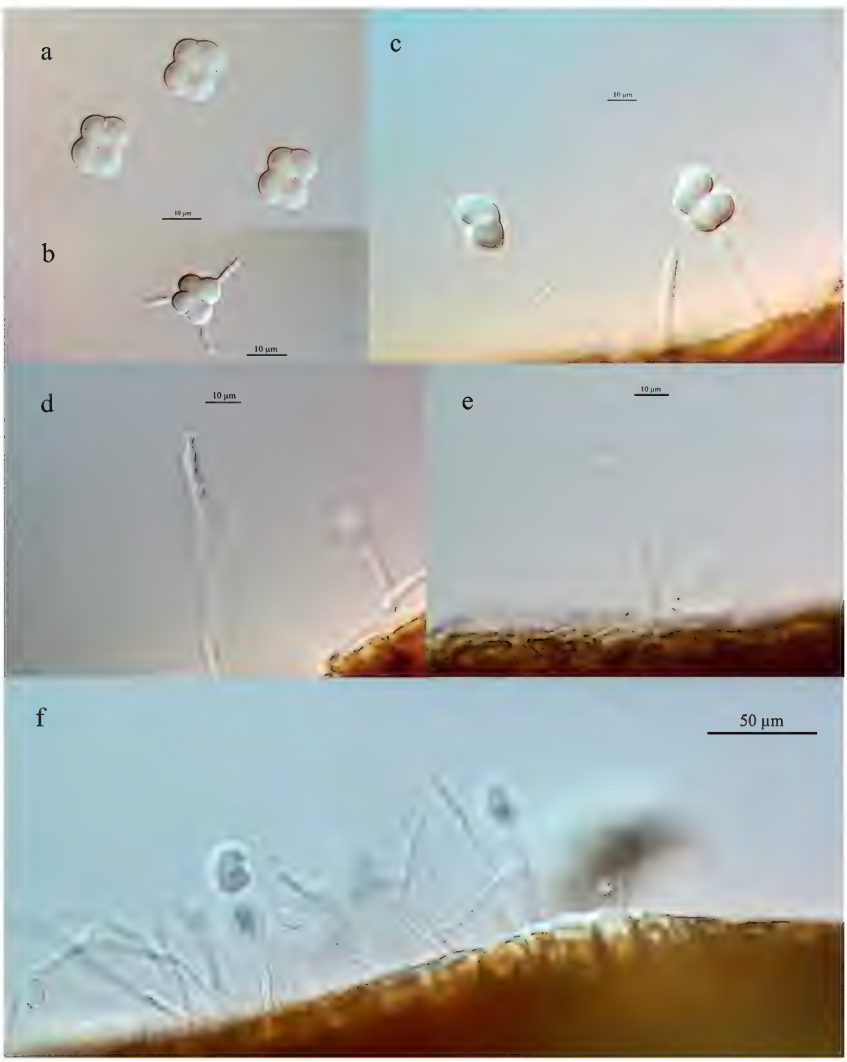

Fig. 1. *Pyramidospora quadricellularis* (holotype, URM 87706): A, B. Conidia. C–F. Conidiophores, conidiogenous cells, and conidia.

Taxonomy

Pyramidospora quadricellularis M.S. Oliveira, Malosso & R.F. Castañeda, **sp. nov.** FIG. 1

MYCOBANK MB 812756

Differs from all other *Pyramidospora* spp. by its 4-celled conidia.

TYPE: Brazil, Pernambuco, Jaboatão dos Guararapes, Refúgio de Vida Silvestre Matas do Sistema Gurjaú, 8°13′S 35°3′W, 85 m alt., on submerged decaying leaves of an unidentified plant in a stream, 13.IV.2015, coll. M.S. Oliveira (**Holotype**, URM 87706).

ETYMOLOGY: Latin, *quadri-* (= four) + *-cellularis* (= celled).

COLONIES on the natural substrate effuse. Mycelium mostly superficial. Hyphae septate, branched, hyaline, 2–2.5 µm diam, smooth. CONIDIOPHORES distinct, erect, straight or decumbent, mostly single, sometimes branched, cylindrical, abruptly attenuated at the apex, 0–7-septate, smooth, ≤50 µm long, 3–4 µm wide. CONIDIOGENOUS CELLS monoblastic, terminal, determinate, integrated in conidiophores and branches, 5–15 × 2.5–3 µm, denticulate with small (1.5–2 µm long) narrow cylindrical teeth. CONIDIA solitary, acrogenous, square rounded to tetrad-shaped or shamrock leaf-shaped, 13–15 × 15–17 µm, hyaline, smooth, sometimes guttulate, composed of 4 globose cells, hyaline, 4–6 µm diam, sometimes with a germ tube.

NOTE: With 4-celled conidia, *P. quadricellularis* is unique and does not resemble any of the described *Pyramidospora* species (TABLE 1; FIGS 2, 3).

TABLE 1. Morphological comparison of conidia of *Pyramidospora* species

SPECIES	SHAPE	BRANCHES	SIZE (µm)	REFERENCE
P. casuarinae	Pyramid-like	6–8	15–20 × 12–18	Nilsson (1962)
P. constricta	Stellate to irregular	2–5	23–47 long	Singh (1972)
P. densa	Stellate	6–8	29–37 wide	Alasoadura (1968)
P. fluminea	Stellate	3–5	16–23 long	Miura & Kudo (1971)
P. herculiformis	H-shaped	4	11–17 long	Singh (1976)
P. quadricellularis	Tetrad-shaped	2	13–15 × 15–17	This paper
P. ramificata	Grape cluster-shaped or irregular	6–7	17–29 long	Miura & Kudo (1971)
P. robusta	Stellate or irregular moniliform cluster	8–13	42–57 long	Moreira & Schoenlein-Crusius (2012)
P. stellata	Stellate	4	15–25 long	Sinclair & Morgan-Jones (1979)

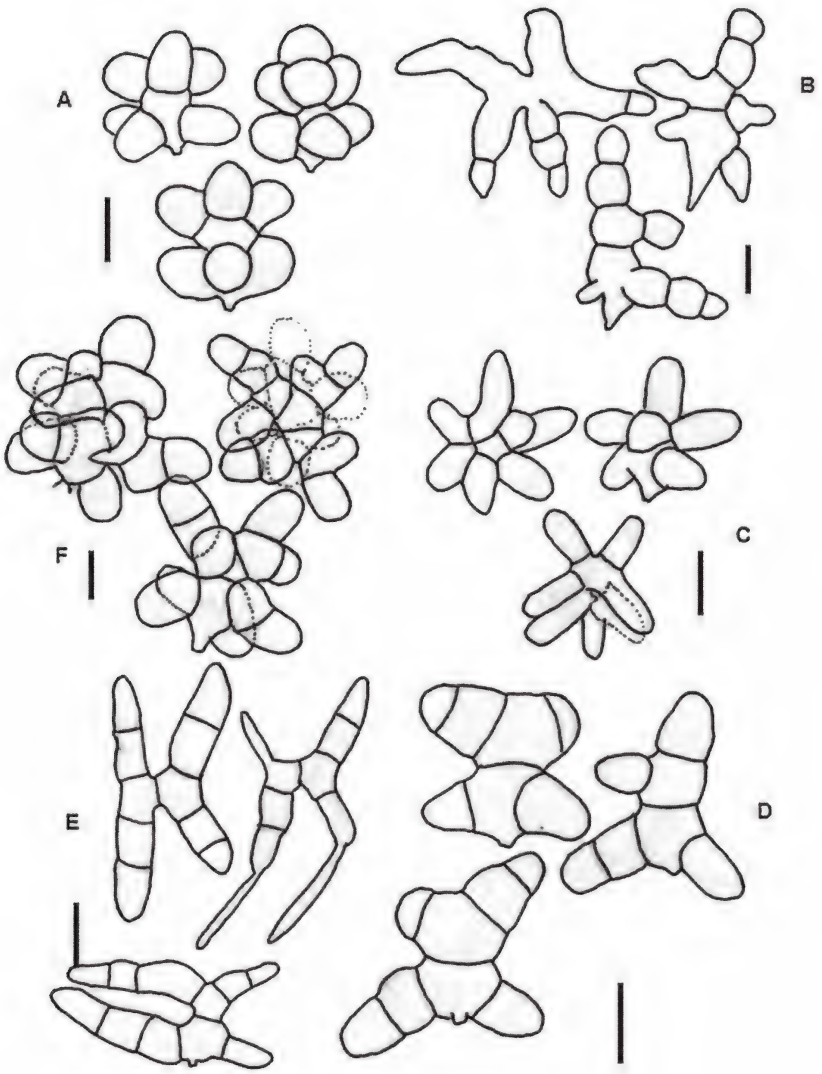

Fig. 2. Conidia of *Pyramidospora* species, redrawn or adapted from original descriptions: A. *P. casuarinae* (Nilsson 1962). B. *P. constricta* (Singh 1972). C. *P. densa* (Alasoadura 1968). D. *P. fluminea* (Miura & Kudo 1971). E. *P. herculiformis* (Singh 1976). F. *P ramificata* (Miura & Kudo 1971). Scale bars = 10 μm.

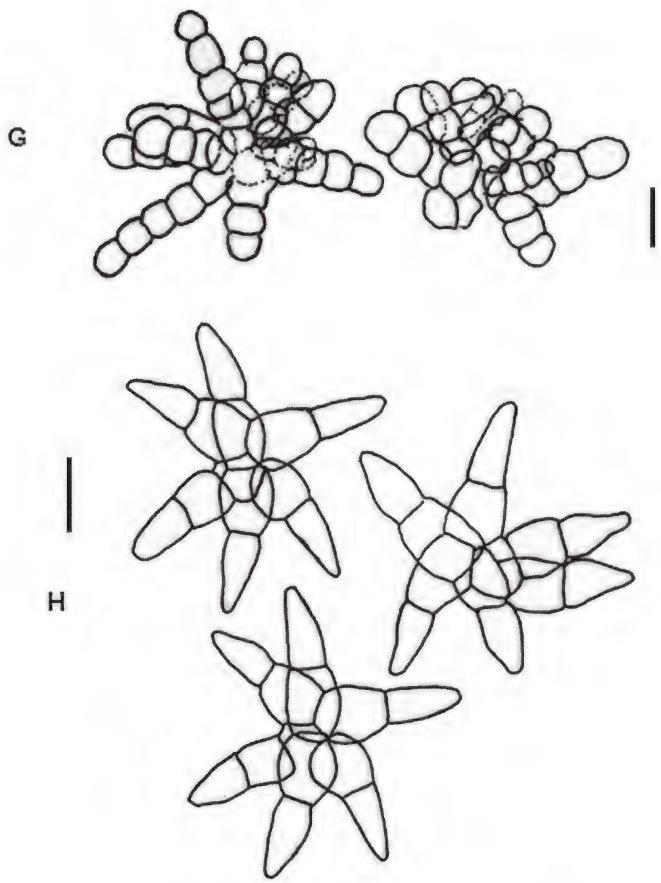

FIG. 3. Conidia of *Pyramidospora* species, redrawn or adapted from original descriptions: G. *P. robusta* (Moreira & Schoenlein-Crusius 2012). H. *P. stellata* (Sinclair & Morgan-Jones 1979). Scale bars = 10 µm.

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Tretolylea, a new genus from the Brazilian semiarid region

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Abstract — *Tretolylea pleiomorpha* gen. & sp. nov. is described and illustrated. It is characterized by inconspicuous prostrate (or decumbent) brown multi-branched conidiophores, tretic conidiogenous cells, blastocatenate cylindrical to oblong euseptate brown conidia, and a *Phialophora*-like synanamorph that arises from the same assimilative hyphae and conidia.

Key words — asexual fungi, sooty moulds, systematics, tropical fungi

Introduction

During investigations of microfungi on dead plant material in the semiarid region of northeast Brazil, an interesting fungus was collected on decaying wood of an unidentified plant. This fungus, which shows remarkable differences from all previously described genera (Seifert et al. 2011), is described as a new genus and species.

Materials & methods

Samples of decaying wood of unidentified woody plants were collected in small separate areas with typical vegetation of the semiarid region and placed in paper bags. In the laboratory the samples were placed in Petri dish moist chambers and stored in a polystyrene box with sterile water plus glycerol at 25°C for 30 days (Castañeda-Ruiz 2005). Mounts of slides were prepared in PVL (polyvinyl alcohol, lactic acid, and phenol), and micrographs were obtained with an Olympus microscope BX 51. The specimen is conserved in the Herbarium, Departamento de Ciências Biológicas, Universidade Estadual de Feira de Santana, Brazil (HUEFS).

Taxonomy

Tretolylea Cantillo, R.F. Castañeda &. Gusmão, **gen. nov.**

MycoBank MB 812409

Differs from *Lylea* by its tretic conidiogenous cells and euseptate conidia.

Type species: *Tretolylea pleiomorpha* Cantillo et al.

Etymology: Greek *treto-* (τρητός) meaning pierced through, referring to the tretic conidiogenous cells, + *-lylea* referring to the hyphomycete genus *Lylea*.

Colonies on the natural substratum effuse, hairy, brown. Mycelium superficial, composed of smooth, septate, brown hyphae. Conidiophores inconspicuous, prostrate or decumbent, multi-septate, brown, resembling assimilative hyphae. Conidiogenous cells terminal, integrated or discrete, determinate and producing one conidium or extending sympodially and producing several conidia sequentially; conidial initiation tretic; conidial secession schizolytic. Conidia blastocatenate, cylindrical to oblong, phragmosporous, euseptate, brown, smooth. Synanamorph *Phialophora*-like arising from the same assimilative hyphae and conidia.

Tretolylea pleiomorpha Cantillo, R.F. Castañeda & Gusmão, **sp. nov.** Figs 1, 2

MycoBank MB 812410

Differs from *Lylea catenulata* by its tretic conidiogenous cells and euseptate conidia.

Type: Brazil, Rio Grande do Norte State: Portalegre, Chapada do Apodí, 6°01′S 37°59′W, 520 m alt., on decaying wood, 3.VIII.2014, coll: T. Cantillo (**Holotype:** HUEFS 211337).

Etymology: Greek, *pleio-* meaning more than usual, + *-morpha*, referring to existing forms of conidium ontogeny.

Colonies on the natural substrate effuse, funiculose, brown. Mycelium superficial, composed of branched, 6–11 µm wide, septate, smooth hyphae. Conidiophores inconspicuous. Conidiogenous cells discrete, cylindrical, determinate and producing one conidium or extending sympodially and producing several conidia sequentially; conidial initiation tretic; conidial secession schizolytic. Conidia blastocatenate, cylindrical to oblong, straight, somewhat curved or slightly, sinuate, rounded at the ends, brown, smooth, 2- to 11-euseptate, slightly darker at septa, 29–47 × 10–19 µm. Synanamorph *Phialophora*-like, conidiophores distinct, branched, conidiogenous cells forming a slightly penicillate cluster, lageniform, discrete, "phialidic" (i.e., producing conidia sequentially by a replacement wall-building apex system from a single fertile locus; Minter et al. 1983), sometimes extending by percurrent enteroblastic proliferation, pale brown, 8–16 × 6–8 µm, with a conspicuous, narrow collarette. Conidia globose, unicellular, subhyaline to pale brown or pale olivaceous-brown, smooth, 2–3 µm diam., accumulating in brown masses.

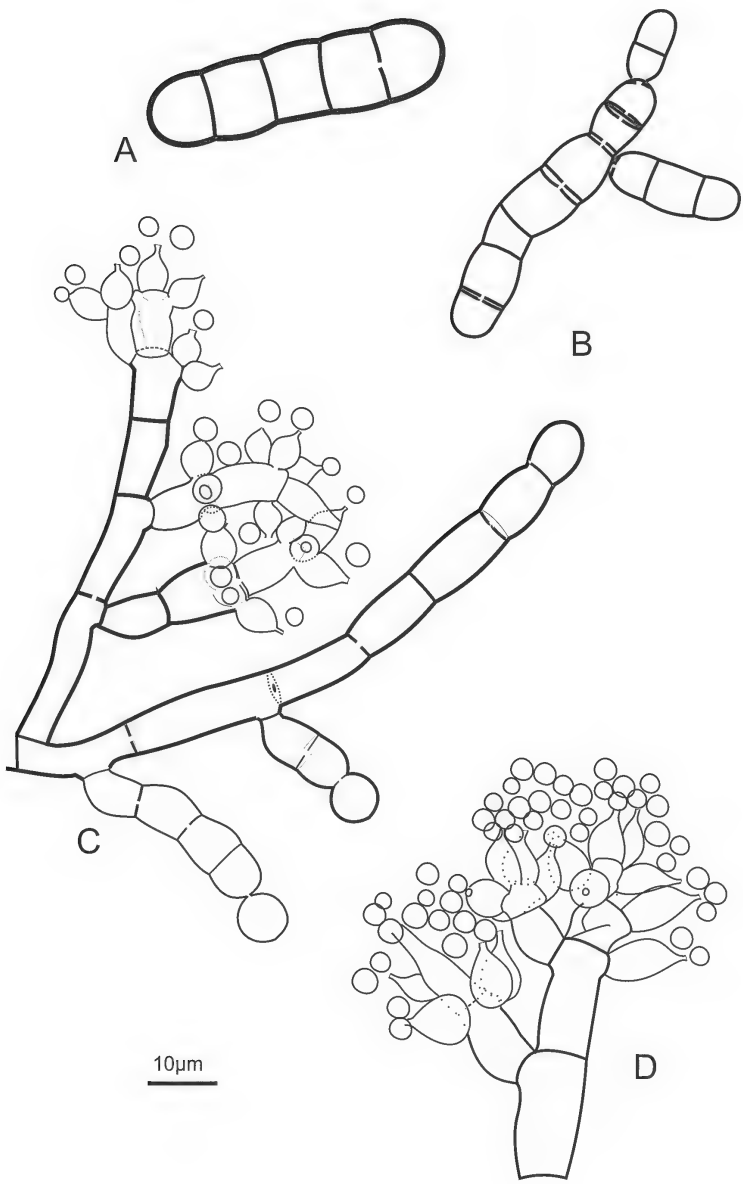

Fig. 1. *Tretolylea pleiomorpha* (holotype, HUEFS 211337).
A, B. Conidia. C. Conidiogenous cells and conidia.
D. Conidiogenous cell and conidia of *Phialophora*-like synanamorph.

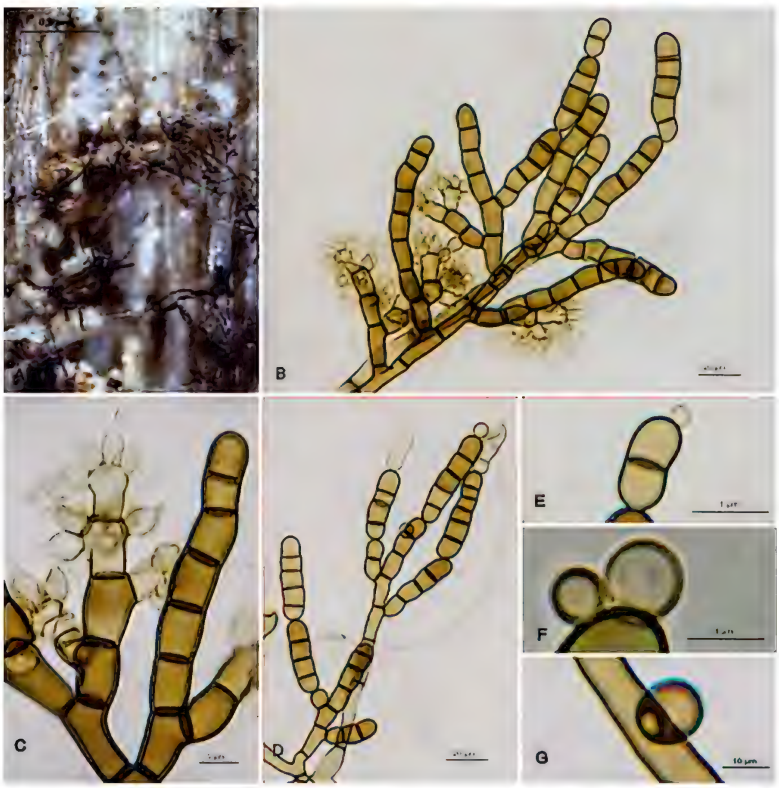

Fig. 2. *Tretolylea pleiomorpha* (holotype, HUEFS 211337). A. Colonies on the natural substratum. B, C. Conidiogenous cells and conidia. D. Conidiogenous cell and conidia. E. Conidial development. F, G. Conidiogenous cells.

Notes: *Lylea* Morgan-Jones superficially resembles *Tretolylea* but has holoblastic conidiogenous cells and distoseptate conidia (Morgan-Jones 1975). *Heteroconium* Petr. (Castañeda-Ruiz et al. 2008, Ma et al. 2012a,b, Ren et al. 2012), *Taeniolella* S. Hughes (Watanabe 1992), and *Taeniolina* M.B. Ellis (Seifert et al. 2011) are similar to *Tretolylea;* these genera produce conidia in unbranched or branched chains, but conidial development is holoblastic. The sooty moulds *Capnophialophora* S. Hughes and *Capnosporium* S. Hughes (Hughes 1976) also superficially resemble *Tretolylea,* but neither has tretic conidial development (Seifert et al. 2011).

This fungus was collected in the semiarid region of Brazil, also known as Caatinga. Caatinga ecosystems are unique to Brazil and are undoubtedly threatened, with only about 1% of the area having formal protection (www.nature.org/ourinitiatives/regions/southamerica/brazil/placesweprotect/caatinga.xml

— accessed 31 May 2015). The type locality is a small remaining fragment of natural or semi-natural vegetation surrounded by farming and other developments. The discovery of this new genus here (and of others already collected by the authors but as yet undescribed) suggests that the Caatinga may be as rich in fungi as in animals and plants, many of which are endemic. As a newly discovered species about which very little is known, the conservation status of *Tretolylea pleiomorpha* is Data Deficient, but if it is found to be an endemic of the region, it is will probably be categorized as threatened.

Acknowledgments

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Acaulospora reducta sp. nov. and *A. excavata* — two glomeromycotan fungi with pitted spores from Brazil

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Abstract — *Acaulospora reducta* sp. nov. and *A. excavata*, both characterized by large pits on their spore surfaces, were found in the semi-humid Atlantic rainforest and semi-arid Caatinga biomes of Northeastern Brazil. Phylogenetic analyses of the ITS regions of the ribosomal gene place the two fungi in two distinct clades within the *Acaulosporaceae*. *Acaulospora reducta* has whitish yellow, dark yellow to light brown spores (135–205 µm in diam.) and irregularly shaped, often edged to sometimes dumbbell-shaped pits (5.5–19 × 3.5–8.6 µm). These large pits have roughened irregular surfaces comprising secondary small pits (c. 0.5 µm broad and deep) and fine ridges. Spores of *A. excavata* are ochre to yellow orange (sometimes yellow) and have regular circular to subcircular pits (4–20 × 4–16 µm) with smooth pit surfaces. Most recently, *A. reducta* was also found in the Cerrado biome of Minas Gerais, suggesting its wide distribution in tropical Brazil.

Key words — *Glomeromycota*, *Diversisporales*, rDNA

Introduction

Recently, the number of new arbuscular mycorrhizal (AM) fungal species forming acaulosporoid (sensu lato) spores has greatly increased (e.g., Goto et al. 2008; Palenzuela et al. 2008, 2011; Błaszkowski 2012; Oehl et al. 2015),

especially in the *Acaulosporaceae* due to concomitant morphological and molecular spore analyses. *Acaulospora* species have been increasingly reported both from colder climates (e.g. Oehl et al. 2011a, 2012; Palenzuela et al. 2013, 2015) and hot tropical areas (e.g., Goto et al. 2010, 2013; Furrazola et al. 2013; Oehl et al. 2014), with some species found in both cold and hot climates (e.g., Oehl et al. 2011b).

During surveys of AM fungal diversity in different agricultural and natural ecosystems in Brazil, two *Acaulospora* species were found with numerous conspicuously large depressions on the spore surfaces. *Acaulospora excavata*, which had been described from a similar climate zone in Ivory Coast (West Africa) and which was easily identified by large regular pits covering the spore surface (Ingleby et al. 1994), had never been subjected to molecular analyses. In *Acaulosporaceae* such prominent pits have rarely been found (e.g., for *A. foveata* Trappe & Janos and *A. bireticulata* F.M Rothwell & Trappe; Rothwell & Trappe 1979, Janos & Trappe 1982 as summarized in Błaszkowski 2012 and Oehl et al. 2012, 2014). The second fungus, new and described and illustrated herein as *Acaulospora reducta,* has similarly large pits that are more irregular and more complex than *A. foveata* and *A. excavata*. The objective of this study was to analyze the two fungi concomitantly on spore morphology and the ribosomal ITS region and to compare both with each other and other *Acaulospora* species.

Material & methods

Study area and sites

Soil samples were collected during the June 2011wet season and subsequent March 2012 dry season at the Itapirema Experimental Station, Agronomic Institute of Pernambuco – IPA, located in the municipality of Goiana (7°38′20″S 34°57′10″W; 13 m asl), Pernambuco State, NE Brazil. *Acaulospora reducta* was found in plantations of cassava (*Manihot esculenta* Crantz; *Euphorbiaceae*), sapodilla (*Manilkara zapota* (L.) P. Royen; *Sapotaceae*), and mahogany (*Swietenia macrophylla* King; *Meliaceae*) of the experimental station, while *A. excavata* was found only in the sapodilla and mahogany plantations. The climate in this sampling area is Ams' (after Köppen; Kottek et al. 2006), semi-humid with a tropical monsoon and a dry summer. Mean annual precipitation is around 2000 mm with a mean annual temperature of 24°C. The soil type is a Ferralsol (IUSS Working Group WRB, 2014). Pereira et al. (2014) describe the soil chemical characteristics; the mean soil pH (H_2O) was 6.1, organic carbon 7.7 g kg^{-1}, and available P 11.0 mg kg^{-1}.

Acaulospora reducta was additionally found in a coastal 'restinga' vegetation in Tamandaré (Pernambuco State; 8°46′52″S 35°06′22″W) and Mataraca (Paraíba State; 6°29′S 34°56′W), in a semiarid area with Caatinga vegetation in Petrolina (Pernambuco State; 9°03′16.68″S 40°18′59.23″W; 389 m asl) and Assú (Rio Grande do Norte State; 5°34′38″S 36°54′30″W; 60 m asl), and in Cerrado Ferralsol soil samples from Sete Lagoas (Minas Gerais State; 19°27′46″S 44°10′24″W; 738 m asl). The Cerrado soil was collected in May 2012 at the Embrapa Maize and Sorghum Experimental Station

from a subsoil layer and used to prepare substrate for "On Farm" AM fungal inoculum production using maize (*Zea mays* L.) and *Urochloa decumbens* (Stapf) R.D. Webster as host plants. Mean soil pH was 5.4 (H_2O) with organic carbon 0.29 g kg^{-1} and available P 0.85 mg kg^{-1}. Lastly, the fungus was also found in Planaltina (near Brasilia) in a no-till production site grown with soybean (Distrito Federal, 15°36′S 47°42′W; 1014 m asl) and in a Sorghum production site near the type location in Itapirema (Pernambuco).

Acaulospora excavata was additionally found in a small home garden in Recife (Pernambuco State; 7°53′06″S and 34°50′11″W) within the rhizosphere of "Palmeira" (*Dypsis lutescens* (H. Wendl.) Beentje & J. Dransf.; *Arecaceae*), a palm known variously as bamboo palm, golden cane palm, areca palm, yellow palm, or butterfly palm.

Arbuscular mycorrhizal cultures

The native AM fungal communities from the sampling sites in Itapirema were propagated in trap cultures over three consecutive 4-month cycles using millet (*Panicum miliaceum* L.), maize, and sunflower (*Helianthus annuus* L.) as hosts in 2 L plastic pots according to Pereira et al. (2014). At the end of each cycle, the plants were allowed to dry and cut off, with 50 g soil aliquots collected for spore extraction. The soil was reseeded for the subsequent cycle. The cultures were maintained at the greenhouse of the Mycology Department, at the Federal University of Pernambuco, Recife, Brazil. However, the two fungi under study did not sporulate within three consecutive propagation cycles.

Morphological analyses

Spores of the two fungi were extracted from the field soil samples by wet sieving and sucrose centrifugation according to Sieverding (1991), separated under a stereomicroscope, and mounted in polyvinyl alcohol–lacto–glycerin (PVLG) and PVLG + Melzer's reagent (Brundrett et al. 1994). Slide mountants were dried (3–5 days at 60°C) to clarify oil drops and observed with a Nikon compound microscope (Eclipse Ni). The spore terminology follows Oehl et al. (2012), Furrazola et al. (2013), and Goto et al. (2013) for acaulosporoid glomeromycotan fungi.

Transmission electron microscopy

The samples of the new fungus were dehydrated by immersion in solutions of increasing concentrations of ethanol (25%, 50%, and 90%) for thirty minutes each followed by three baths with 100% ethanol. The samples were then immersed in three 30-minute baths in 100% acetone. Dehydrated spores critically point dried in an Emitech K850, sputter coated with gold in an Emitech K550, and examined under a Zeiss DSM 940 A scanning electron microscope at an accelerating voltage of 5–15 kV. The analyses were carried out at the Nucleus of Applied Biology microscopy lab at Embrapa Maize and Sorghum.

Molecular analyses

After isolation from field soil samples, spores from the two fungi were washed in ultrapure water and sonicated three to four times. The DNA was extracted from individual spores placed on a slide in a drop (5–10 µl) of ultrapure water, and crushed with a sterile needle. Crude DNA extract was used as template for a semi-nested PCR using the primers ITS5+LSUAC (White et al. 1990; 5′-CCATTACGTCAGCATCCTTAGCG-3′) and ITS1+28G6 (White et al. 1990; 5′-CGGGATTCTCACCCTCTATG-3′) consecutively.

PCR reactions were carried out in a volume of 50 µl, containing 75 mM Tris-HCl pH 8.8, 200 mM $(NH_4)_2SO4$, 0.01% Tween 20, 2 mM $MgCl_2$, 200 µM each dNTPs, 1 µM of each primer, and 2 units of DreamTaq DNA polymerase (Thermo Scientific, Maryland, USA); cycling parameters were one cycle of 5 min at 95°C; 40 cycles of 45 s at 94°C, 1 min at 55°C, and 1 min at 72°C; and a final elongation of 7 min at 72°C following the last cycle. The amplified products (~600) were purified with a GeneJET PCR Purification Kit (Thermo Scientific, USA) and cloned with a CloneJET PCR Cloning kit (Thermo Scientific, USA) following the manufacturer's instruction, and then sequenced by the Central Laboratory of the CCB/UFPE (Recife, Brazil). The phylogeny was inferred by ITS sequence analyses. These AM fungal sequences were then aligned with other glomeromycotan sequences from GenBank in ClustalX (Larkin et al. 2007). Sequences from *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüssler were used as outgroup. Prior to phylogenetic analysis, the model of nucleotide substitution was estimated using Topali 2.5 (Milne et al. 2004). Bayesian (two runs over 3×10^6 generations with a burn in value of 25%) and maximum likelihood (1000 bootstrap) analyses were performed, respectively, in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) and PhyML (Guindon & Gascuel 2003) launched from Topali 2.5, using the GTR + I model. Maximum parsimony analysis was performed using PAUP*4b10 (Swofford 2003) with 1000 bootstrap replications.

Taxonomy

Acaulospora reducta Oehl, B.T. Goto & C.M.R. Pereira, **sp. nov.** FIGS 1–11

MYCOBANK MB 812332

Differs from *Acaulospora excavata* by its more irregular pits on the outer spore wall that have roughened and slightly pitted inner surfaces.

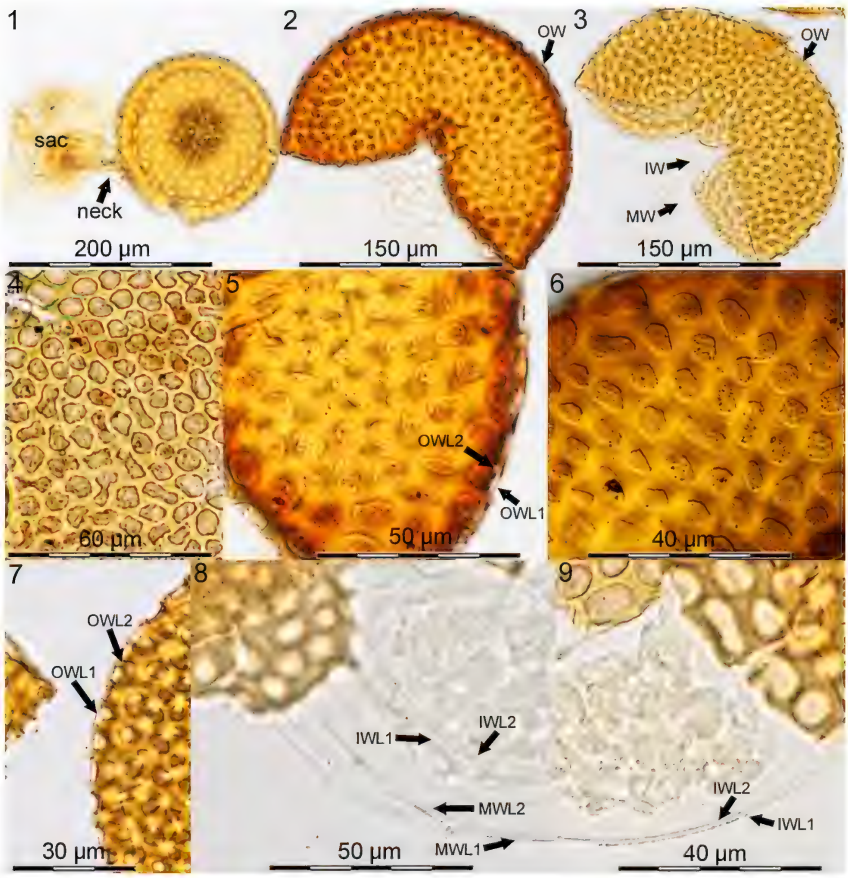
TYPE: Brazil, Pernambuco State, Goiana, Experimental Station of Itapirema (Instituto Agronômico de Pernambuco), from a Ferralsol in a cassava plantation, 1.6.2011, coll. C.M.R. Pereira (**Holotype**, URM87697; **isotypes**, URM87698, ZT Myc 55894; GenBank KM057063–KM057067).

ETYMOLOGY: *reducta* (Latin), referring to the small depressions within the large pits on the outer spore wall.

SPOROCARPS unknown. Acaulosporoid spores form singly in soils laterally on the tapering hyphal neck of sporiferous saccules.

SPORIFEROUS SACCULES are hyaline to subhyaline to light yellow, rarely dark yellow, globose to subglobose, 140–210 µm in diam. and form terminally on hyphae. They are generally detached from mature spores. The saccule wall generally is bi-layered with a rapidly degrading evanescent hyaline to light yellow outer layer (0.5–1.5 µm) and a semi-persistent sub-hyaline to light yellow to dark yellow inner layer, which is 1.8–2.8(–4.4) µm.

SPORES form singly on the saccule necks. They are globose (135–205 µm diam.) to subglobose to oval (145–210 × 130–195 µm), whitish yellow to dark yellow. The spores form three walls: outer wall (OW), middle wall (MW), and inner wall (IW).



Figs 1–9. *Acaulospora reducta*. 1. Spore formed laterally on the neck of a sporiferous saccule (sac); globose saccule terminus already collapsed; 2–3. Crushed spores with three walls: pitted outer wall (OW), smooth middle wall (MW) and granular inner wall (IW); 4–6. Large, irregular pits on the outer OW surface in planar view showing a roughened, irregular inner surface structure resembling small depressions and projections of c. 0.5–1.5 µm in diam. and depth; 7. Pits on OW in cross view; OW with two layers visible (OWL1–2); inner, small pits not clearly visible within the large depressions; 8–9. MW and IW with multiple layers visible (MWL1–2; IWL1–2).

Outer wall consists of three layers, with the outermost layer (OWL1) evanescent to semi-persistent, 1.5–2.6 µm thick, hyaline to subhyaline. In older spores, the outer wall layer (OWL1) might degrade completely, but usually some fragments remain on the OWL2 surface. OWL2 is laminated, 6.0–14.5 µm thick, whitish yellow, dark yellow to light brown and has innumerous irregularly-shaped, often edged to sometimes dumbbell-shaped pits (5.5–19 ×

3.5–8.6 µm) that itself have roughened surfaces consisting of small depressions of approx. 0.5–1.5 µm in diam. and 0.5–1.5 µm depth) surrounded by projections of similar dimensions to fine ridges. OWL3 is thin (0.7–1.2 µm), yellowish and tightly attached to OWL2. None of the OW layers stains in Melzer's reagent.

Middle wall is bi-layered, hyaline, semi-flexible to flexible and in total 2.0–3.5 µm thick. MWL1 is 1.0–1.7 µm thick, and generally tightly adherent to MWL2, which is 1.0–1.8 µm. None of the MW layers stains in Melzer's reagent.

Inner wall is three-layered, hyaline, and in total 3.6–5.8(–9.2) µm thick. The outer layer IWL1 is 0.6–1.3 µm, with a granular ('beaded') structure, and tightly adherent to IWL2, which is 2.5–3.5 µm thick and sometimes, as being 'amorphous', expands up to 6.9 µm in lactic acid based mountants. IWL3 tightly adheres to IWL2, and is, as only 0.5–1.0 µm thick, usually difficult to detect. IWL2 stains dark red purple in Melzer's reagent, but this stain may disappear completely within months in the mountants.

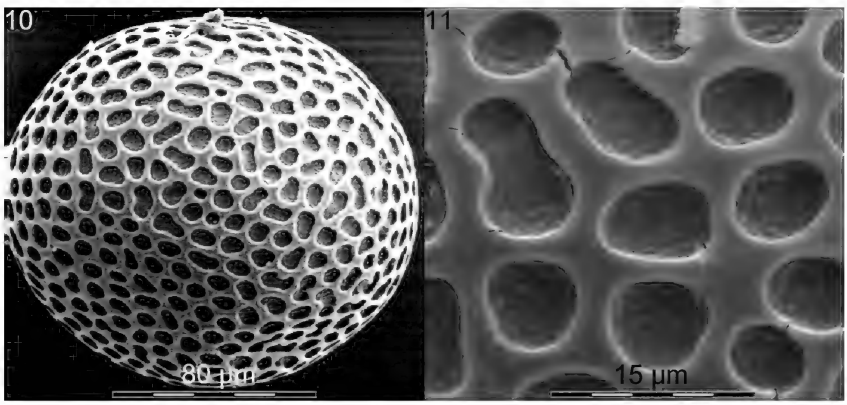
Germination was not observed.

Arbuscular mycorrhiza formation so far unknown.

Additional specimens examined: **BRAZIL**, **Pernambuco State**, Goiana, Experimental Station of Itapirema, from a sapodilla plantation, 1.6.2011, coll. C.M.R. Pereira (URM87699); from a mahogany plantation, 1.6.2011, coll. C.M.R. Pereira (URM87700); from a *Sorghum bicolor* production site, coll. D.M.A. Assis, 18.8.2015 (URM88218); Tamandaré, from a 'restinga' vegetation (1.6.2013, coll. D.K.A. Silva (URM88213); Petrolina, from a natural Caatinga, 1.6.2014, coll. D.K.A. Silva (URM88214); **Paraíba State**, Mataraca, from a 'restinga'; 1.6.2012, coll. D.K.A. Silva (URM88215); **Rio Grande do Norte State**, Assú, from a natural Caatinga, 15.9.2013, coll. A.S. Medeiros (URM88216). **Minas Gerais State**, Sete Lagoas, from a natural Cerrado forest savanna, 1.5.2012, coll. F.A. Souza (URM88217, ZT Myc 55273); **Distrito Federal**, Planaltina, from a no-till production site with soybean, 1.3.2014, coll. J.S. Pontes (URM88219).

Phylogenetic analyses — rDNA sequence analysis of the ITS region inferred the phylogeny of *Acaulosporaceae,* placing *A. reducta* in a monophyletic clade with high levels of support near *A. scrobiculata* and *A. minuta* and supporting *A. reducta* as a new species within *Acaulospora* (Fig. 12). In BLASTn analysis, the species most closely related to *A. reducta* was *A. scrobiculata* (FR692353) with 97% identity; no environmental ITS sequences deposited in GenBank corresponded to *A. reducta*.

Distribution — *Acaulospora reducta* has been recorded from the semi-humid Atlantic rainforest, the semi-arid Caatinga, and the Cerrado savanna biomes of tropical Brazil. In the Atlantic Rainforest biome, the fungus was isolated from agricultural systems in Itapirema (Goiana, Pernambuco State) and coastal sand dunes with typical 'restinga' vegetation in Tamandaré (Pernambuco State) and Mataraca (Paraíba State). In the Caatinga biome, it was found in regeneration areas from the National Forest Flona de Açu in Assú



Figs 10–11. *Acaulospora reducta* (transmission electron microscopy). 10. Uncrushed spore with innumerous large pits on the spore surface and multiple small depressions and projections on the pit surfaces; 11. Spore segment showing detail view of the multiple small depressions and projections within the large pits.

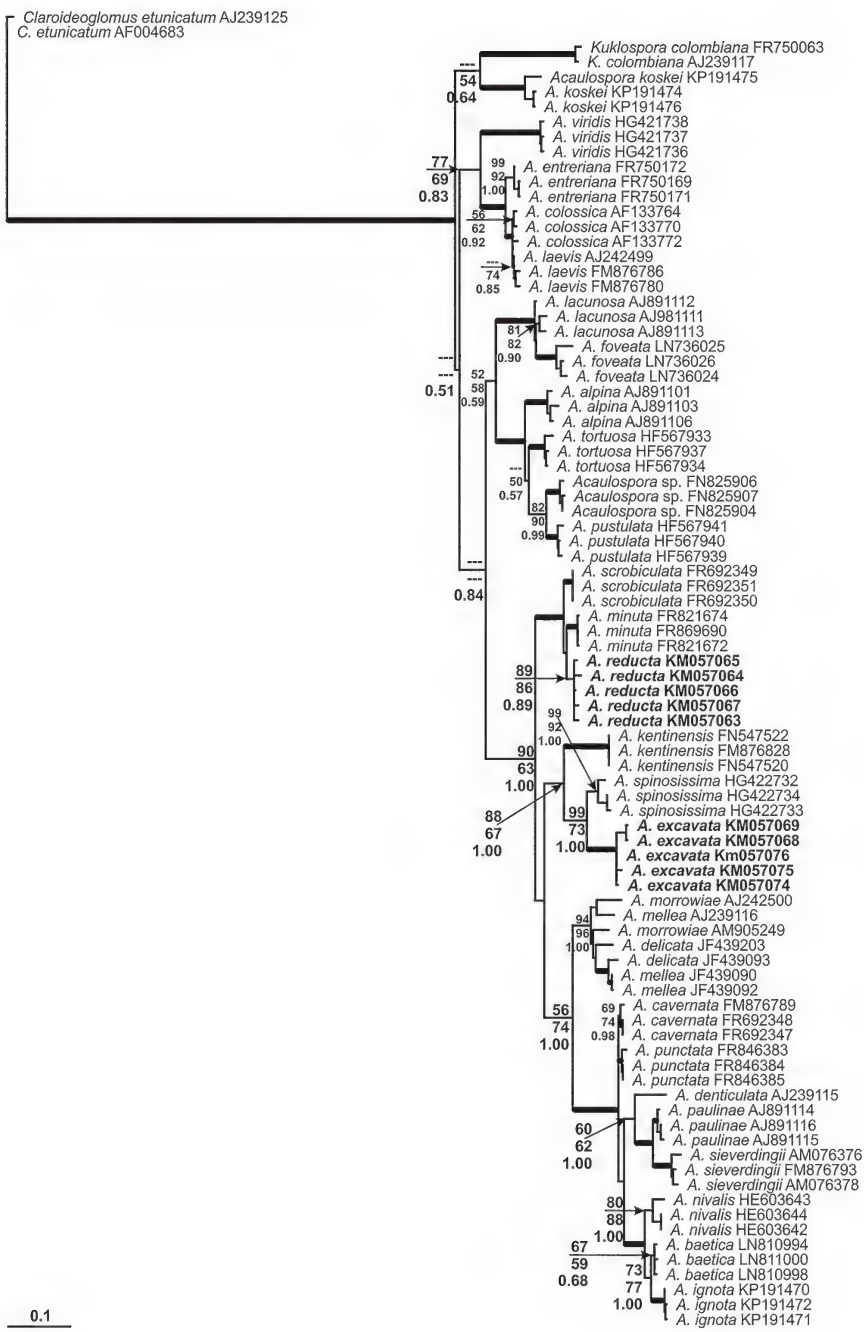
(Rio Grande do Norte State) and in a preserved Caatinga area in Petrolina (Pernambuco State). Finally, in the Cerrado biome it was found in a forest savanna of Sete Lagoas (Minas Gerais State). It was also found in mycorrhizal inoculum production cultures of non-sterile soils, derived from this Cerrado area and cultivated with maize and *Urochloa decumbens*. The fungus co-occurred at the type location with *A. excavata*, among many other AMF species (see Pereira et al. 2014).

Acaulospora excavata Ingleby & C. Walker, Mycotaxon 50: 100. 1994 Figs 13–16

MycoBank MB 361679

Spores form laterally on the necks of sporiferous saccules, which are 110–190 × 100–165 µm. Spores are globose to subglobose to oval (115–195 × 100–180 µm), pale ochraceous to pale yellow to yellow orange to sometimes bright yellow. The spores form three walls: triple-layered outer wall (OWL1-3), bi-layered middle wall (MWL1-2), and triple-layered inner wall (IWL1-3). OWL2 with circular to elliptical pits (3.9–20 × 3.5–16 µm in diam.; 2.2–6.3 µm in depth). IWL1 with 'beaded', granular structure; IWL2 staining purple to dark purple in Melzer's reagent.

Specimens examined — **COTE D'IVOIRE**, southwest of Tissale, Mopri Forest Reserve, under *Terminalia ivorensis*, 30 Oct 1990, C. Walker 1674 (OSC 83345, isotype). **BRAZIL**, **Pernambuco State**, Goiana, Experimental Station of Itapirema (Instituto Agronômico de Pernambuco), from a Ferralsol in a sapodilla plantation, 1.6.2011, coll.: C.M.R. Pereira (URM87701), and from a mahogany plantation, 1.6.2011, coll.: C.M.R. Pereira (ZT Myc 55895); **Pernambuco State**, Recife, in the rhizosphere of


Claroideoglomus etunicatum AJ239125
C. etunicatum AF004683
Kuklospora colombiana FR750063
K. colombiana AJ239117
Acaulospora koskei KP191475
A. koskei KP191474
A. koskei KP191476
A. viridis HG421738
A. viridis HG421737
A. viridis HG421736
A. entreriana FR750172
A. entreriana FR750169
A. entreriana FR750171
A. colossica AF133764
A. colossica AF133770
A. colossica AF133772
A. laevis AJ242499
A. laevis FM876786
A. laevis FM876780
A. lacunosa AJ891112
A. lacunosa AJ981111
A. lacunosa AJ891113
A. foveata LN736025
A. foveata LN736026
A. foveata LN736024
A. alpina AJ891101
A. alpina AJ891103
A. alpina AJ891106
A. tortuosa HF567933
A. tortuosa HF567937
A. tortuosa HF567934
Acaulospora sp. FN825906
Acaulospora sp. FN825907
Acaulospora sp. FN825904
A. pustulata HF567941
A. pustulata HF567940
A. pustulata HF567939
A. scrobiculata FR692349
A. scrobiculata FR692351
A. scrobiculata FR692350
A. minuta FR821674
A. minuta FR869690
A. minuta FR821672
A. reducta KM057065
A. reducta KM057064
A. reducta KM057066
A. reducta KM057067
A. reducta KM057063
A. kentinensis FN547522
A. kentinensis FM876828
A. kentinensis FN547520
A. spinosissima HG422732
A. spinosissima HG422734
A. spinosissima HG422733
A. excavata KM057069
A. excavata KM057068
A. excavata Km057076
A. excavata KM057075
A. excavata KM057074
A. morrowiae AJ242500
A. mellea AJ239116
A. morrowiae AM905249
A. delicata JF439203
A. delicata JF439093
A. mellea JF439090
A. mellea JF439092
A. cavernata FM876789
A. cavernata FR692348
A. cavernata FR692347
A. punctata FR846383
A. punctata FR846384
A. punctata FR846385
A. denticulata AJ239115
A. paulinae AJ891114
A. paulinae AJ891116
A. paulinae AJ891115
A. sieverdingii AM076376
A. sieverdingii FM876793
A. sieverdingii AM076378
A. nivalis HE603643
A. nivalis HE603644
A. nivalis HE603642
A. baetica LN810994
A. baetica LN811000
A. baetica LN810998
A. ignota KP191470
A. ignota KP191472
A. ignota KP191471
0.1

Dypsis lutescens, 1.7.2012, coll.: I.P. de Monte Júnior (URM87702, GenBank KM057068–KM057069; KM057074–KM057076).

Notes — Our morphological analyses revealed no differences between the type specimens of *A. excavata* isolated from Mopri Forest Reserve in Ivory coast (West Africa) and our specimens isolated from Goiana and Recife, in Northeastern Brazil (Figs 13–16). Our study is the first to present molecular phylogenetic data for *A. excavata* (Fig. 12).

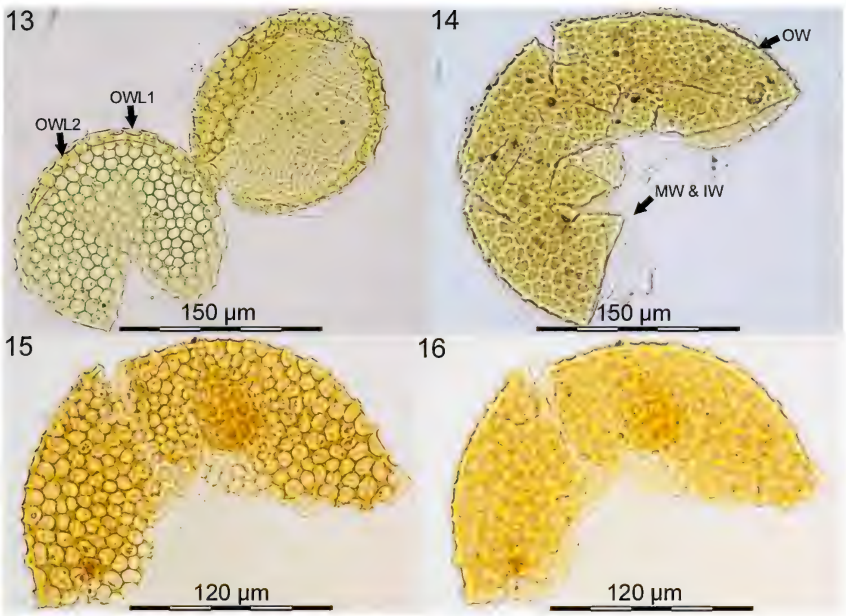
Phylogenetic analyses — The ITS sequence analysis grouped *A. excavata* with *A. spinosissima* and *A. kentinensis*. In BLASTn analysis, the sequences closest to *A. excavata* were from *A. spinosissima* (HG422733–HG422734) with 94% identity. No environmental ITS sequences deposited in GenBank corresponded with *A. excavata*.

Discussion

Acaulospora reducta and *A. excavata* represent two species with characteristic large pits on the outer spore wall that easily distinguish them from all other species in the *Acaulosporaceae*. While *A. excavata* has somewhat regularly sized pits (concerning both diameter and depth) and smooth inner pit surfaces, *A. reducta* has more irregular and often edged pits with roughened surfaces consisting of small depressions and projections inside the large pits, so that the pits are doubly ornamented, a diagnostic character within the *Glomeromycota*.

There are only a few other *Acaulospora* species that have pits close to as large as those found in *A. reducta* and *A. excavata*, such as *A. bireticulata*, *A. foveata*, and *A. verna* (Rothwell & Trappe 1979, Janos & Trappe 1982, Błaszkowski 2012). *Acaulospora foveata* has pitted spores similar to *A. excavata*, but its spores are reddish brown to brownish black and generally larger than those of *A. reducta* and *A. excavata* (Janos & Trappe 1982, Ingleby et al. 1994), and the pits are generally more irregular in *A. foveata* than in *A. excavata* (Trejo et al. 2015). *Acaulospora bireticulata* has light brown to brown spores with a double reticulum on the outer OW surface and regular and conspicuous spines within the large pits (Rothwell & Trappe 1979, Walker & Trappe 1981). *Acaulospora verna* also has rather large pits (3.2–7.8 µm if circular to subcircular, or 3.2–4.2 × 9.8–16.2 µm if ellipsoidal to oblong), but its spores are clearly smaller (60–95 µm) than those of *A. reducta* and the pit surfaces are smooth (Błaszkowski 2012).

Fig. 12. Phylogenetic tree of the *Acaulosporaceae* obtained by analyses from ITS1, 5.8 rDNA, and ITS2 sequences of different *Acaulospora* spp. Sequences are labeled with their database accession numbers. Support values (from top) are from maximum parsimony (MP), maximum likelihood (ML), and Bayesian analyses. Sequences obtained in this study are in boldface. Only support values of at least 50% are shown. Thick branches represent clades with more than 95% of support in all analyses. The tree was rooted by *Claroideoglomus etunicatum*. (Consistency Index = 0.50; Retention Index = 0.86).



Figs 13–16. *Acaulospora excavata.* Crushed spores with three walls: pitted outer wall (OW), smooth middle wall (MW) and granular inner wall (IW); large pits on the outer OW surface, with smooth surfaces within the pits. 13–14. OSC 83345, Côte d'Ivoire; 15. URM87701, Brazil; 16. URM87702, Brazil.

Other species (e.g., *A. cavernata,* Błaszkowski 1989; *A. elegans,* Gerdemann & Trappe 1974) have significantly smaller pits than *A. reducta* and *A. excavata*.

Phylogenetically, *A. reducta* and *A. excavata* form two distinct clades within *Acaulosporaceae*. Interestingly, they do not group closely together (Fig. 12) nor with *A. foveata,* which was recently analyzed from its type location in Veracruz, Mexico (Trejo et al. 2015). It would nonetheless be worthwhile to analyze also *A. excavata* from its type location in Ivory Coast, West Africa (Ingleby et al. 1994), from a climatic zone comparable to semi-humid tropical South America. Its distinctive morphology (Figs. 13–16), however, does support our species identification of the *A. excavata* isolates from Pernambuco (Brazil).

Acaulospora reducta is known from three biomes in NE and SE Brazil, the semi-humid Atlantic rainforest, the semi-arid Caatinga and the Cerrado savanna biomes. These findings suggest that *A. reducta* is widely distributed in tropical Brazil. The morphology of *A. excavata*, originally described from tropical West Africa, supports its occurrence in tropical South America. In the

present study, molecular phylogenetic data of this fungus were shown for the first time.

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Lecanicillium uredinophilum sp. nov. associated with rust fungi from Korea

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Abstract — A novel species of *Lecanicillium* found on rust fungi is described. *Lecanicillium uredinophilum* sp. nov. is closely related to *L. longisporum* but differs morphologically in having longer phialides. Multiple gene sequence analysis supports its taxonomic position in the genus *Lecanicillium*.

Key words — *Cordycipitaceae*, fungicolous species, hyperparasitism, *Verticillium*-like hyphomycete

Introduction

The genus *Lecanicillium*, typified by *L. lecanii*, was established to accommodate entomogenous or fungicolous species that had been previously assigned to *Verticillium* sect. *Prostrata* (Gams & Zare 2001). Subsequently, Zare & Gams (2001) described 14 *Lecanicillium* species based on a combination of morphological characteristics and analysis of the internal transcribed spacer (ITS) rDNA sequence. Subsequently, nine additional taxa have been placed in *Lecanicillium* (Kope & Leal 2006, Zare & Gams 2008, Sukarno et al. 2009, Kaifuchi et al. 2013). Phylogenetically, the genus *Lecanicillium* is placed in *Cordycipitaceae* Kreisel ex G.H. Sung et al. (Sung et al. 2007). The genus is characterized by elongated conidia and simple phialides that are held in whorls or singly, arising from prostrate aerial hyphae. Sung et al. (2007) demonstrated that the genus *Lecanicillium* is not monophyletic but paraphyletic, a fact that was inferred from multigene phylogenetic analysis using the nuclear ribosomal

small and large subunits (SSU, LSU), translation elongation factor 1-α (TEF1), and the largest and second largest subunits of RNA polymerase II (RPB1, RPB2). More recently, Kouvelis et al. (2008) showed that phylogenetic analysis, using the three mitochondrial genes and the ITS region combined, placed some taxonomically uncertain isolates of *Verticillium* species into *Lecanicillium*.

During extensive surveys on the occurrence of hyperparasitic fungi attacking rusts in Korea, three isolates of a *Lecanicillium*-like fungus were obtained from the rust sori. In this study, these fungi are characterized based on their in vivo and in vitro morphology. Furthermore, the phylogenetic positions of the isolates have been investigated by sequence analysis of the combined genes SSU, LSU, TEF1, RPB1, and RPB2. As a result, these isolates are identified as representing a new species of *Lecanicillium*.

Materials & methods

Isolates

Three collections of rust infected with *Lecanicillium* were collected from several localities of Korea during 2008–2009. Single spore isolates from fresh materials were successfully obtained and grown on PDA at room temperature for 4 weeks in the dark. The isolates were deposited in the Korean Agricultural Culture Collection (KACC) of the National Academy of Agricultural Science, Wanju, Korea. Voucher specimens are housed in the Korea University herbarium, Seoul, Korea (KUS). For this study, ex-type strains and reference isolates of *Lecanicillium* species were gathered from the Centraalbureau voor Schimmelcultures (CBS, the Netherlands) and analyzed together with the Korean isolates. Detailed information on all the isolates used is shown in Table 1.

Morphological study

Fungal structures from air-dried specimens were mounted in 3% KOH solution, whereas those from monoconidial isolates were mounted in sterilized water. They were measured at magnifications of 400× and 1000×, using an eyepiece micrometer and a model BX51 microscope (Olympus, Tokyo, Japan). Microscopic images were photographed using an Axio imager microscope (Carl Zeiss, Göttingen, Germany).

Molecular phylogenetic analysis

DNA extraction, PCR amplification, and sequencing — For genomic DNA extraction, mycelial mats and conidia of the *Lecanicillium* isolates were harvested by scraping the surface of colonies that had been grown on PDA plates for one month. Total genomic DNA was prepared according to the method of Lee & Taylor (1990). PCR amplification was performed for the SSU rDNA using primers NS1 and NS4 (White et al. 1990), and for the LSU rDNA using primers LROR and LR7 (Vilgalys & Hester 1990). The PCRs for these two regions of rDNA were conducted as follows: an initial denaturation for 5 min at 95°C, followed by 35 cycles of denaturation for 1 min at 95°C, annealing for 1 min at 58°C, extension for 2 min at 72°C, and a final extension for 10 min at 72°C. A fragment of the TEF1 gene was amplified using the primer pair EF1-983F

Table 1. *Lecanicillium* and *Simplicillium* sequences used in phylogenetic analyses.

Taxon	Isolate	GenBank Accession No.				
		SSU	LSU	TEF1	RPB1	RPB2
L. uredinophilum	KACC 44082	KM283758	KM283782	KM283806	KM283828	KM283848
	KACC 47756	KM283759	KM283783	KM283807	KM283829	KM283849
	KACC 44066	KM283760	KM283784	KM283808	KM283830	KM283850
L. acerosum	CBS 418.81^{T}	KM283762	KM283786	KM283810	KM283832	KM283852
L. antillanum	CBS 350.85^{T}	AF339585	AF339536	DQ522350	DQ522396	DQ522450
L. aphanocladii	CBS 797.84^{T}	KM283763	KM283787	KM283811	KM283833	KM283853
L. aranearum	CBS 726.73A	AF339586	AF339537	EF468781	EF468887	EF468934
L. attenuatum	CBS 402.78	AF339614	AF339565	EF468782	EF468888	EF468935
L. dimorphum	CBS 345.37	KM283764	KM283788	KM283812	KM283834	KM283854
	CBS 363.86^{T}	AF339608	AF339559	EF468784	EF468890	—
L. flavidum	CBS 300.70D	KM283765	KM283789	KM283813	—	KM283855
	CBS 342.80^{T}	KM283766	KM283790	KM283814	—	—
L. fungicola var. *aleophilum*	CBS 357.80^{T}	KM283767	KM283791	KM283815	KM283835	KM283856
L. fungicola var. *fungicola*	CBS 992.69^{T}	KM283768	KM283792	KM283816	—	KM283857

Table 1 concluded on p. 1000

Table 1, concluded

Taxon	Isolate	GenBank Accession No.				
		SSU	LSU	TEF1	RPB1	RPB2
L. fusisporum	CBS 162.70	KM283769	KM283793	KM283817	KM283836	KM283858
	CBS 164.70^{T}	AF339598	AF339549	EF468783	EF468889	—
L. lecanii	CBS 101247	KM283770	KM283794	DQ522359	KM283837	KM283859
	CBS 102067^{T}	KM283771	KM283795	KM283818	KM283838	KM283860
L. longisporum	CBS 102072	KM283772	KM283796	KM283819	KM283839	KM283861
	CBS 126.27^{T}	KM283773	KM283797	KM283820	KM283840	KM283862
L. muscarium	CBS 143.62^{T}	KM283774	KM283798	KM283821	KM283841	KM283863
L. pissodis	CBS 118231^{T}	KM283775	KM283799	KM283822	KM283842	KM283864
L. psalliotae	CBS 367.86	KM283776	KM283800	KM283823	—	—
L. saksenae	CBS 532.81^{T}	AF339609	AF339560	EF469067	EF469096	EF469112
Lecanicillium sp.	CBS 639.85	KM283777	KM283801	KM283824	KM283843	KM283865
L. tenuipes	CBS 309.85	KM283778	KM283802	DQ522341	KM283844	KM283866
L. wallacei	CBS 101237^{T}	AY184978	AY184967	EF469073	EF469102	EF469119
Simplicillium lanosoniveum	CBS 101267	AF339603	AF339554	DQ522357	DQ522405	DQ522463

and EF1-2218R (Rehner 2001). The PCR cycling conditions were as follows: an initial denaturation for 2 min at 96°C, followed by 35 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 60°C, extension for 30 s at 72°C, and a final extension for 7 min at 72°C. A region of the RPB1 gene was amplified with the primer pair CRPB1 and RPB1-Cr (Castlebury et al. 2004). The PCR cycling conditions used were as those described by Castlebury et al. (2004). A portion of the RPB2 gene was amplified with the primer pair fRPB2-5F and fRPB2-7cR (Liu et al. 1999). The PCR cycling conditions were as follows: an initial denaturation for 5 min at 94°C, followed by 35 cycles of denaturation for 1 min at 94°C, annealing for 10 s at 55°C, extension for 2 min at 72°C, and a final extension for 10 min at 72°C. The PCR amplicons were purified using MultiScreen HTSTM PCR filter plates (Millipore, Carrigtwohill, Cork, Ireland). Purified amplicons were directly sequenced on an ABI Prism™ 377 DNA sequencer (Applied Biosystems, Foster City, CA, USA) with the same primer pairs used for the PCR amplification.

Sequence alignment and phylogenetic analysis — All resulting sequences were edited using DNAStar Lasergene Seqman™ and EditSeq™ software version 7.1.0 (DNAStar, Madison, WI, USA). Reference gene sequences were retrieved from GenBank. The RPB1 and PRB2 gene sequences were unobtainable for a few isolates and thus were treated as missing data in the phylogenetic analysis, and the areas of ambiguity in the alignments were excluded. Multiple sequence alignments were performed using MAFFT version 6.864 (Katoh et al. 2005) with the G-INS-i strategy. The alignments were manually adjusted, if needed. A phylogenetic tree was reconstructed from the Bayesian inference analysis. The model of DNA substitution was estimated with jModelTest version 0.1.1 (Posada 2008). The Bayesian inference analysis was performed using MrBayes version 3.0b4 (Ronquist & Huelsenbeck 2003). Four incrementally heated simultaneous Markov chains were run for one-million generations, with a tree saved at every 100th generation. The first 1000 trees generated from this analysis were discarded. A majority rule consensus tree showing all compatible partitions was obtained with the posterior probability (PP) for each group. Only PP values ≥50% were indicated in the resulting tree. Branch lengths were computed as the mean values over the trees sampled. The phylogenetic trees were viewed with TreeViewX version 0.5.0 (Page 1996). A sequence of *Simplicillium lanosoniveum* (J.F.H. Beyma) Zare & W. Gams (CBS 101267) served as outgroup for the multigene phylogenetic analysis.

Results

Molecular phylogenetic analysis

The resulting alignment included the sequences of the five genes from 27 *Lecanicillium* isolates and one outgroup isolate. The alignment consisted of 3999 characters (including alignment gaps) that comprised 952 characters of SSU, 759 of LSU, 871 of TEF1, 522 of RPB1, and 895 of RPB2. A majority rule consensus tree (Fig. 2) was generated from the Bayesian analysis, using the GTR + Γ + I model selected by jModelTest. The three Korean isolates found on uredinia of the rust fungi *Pucciniastrum agrimoniae* and *Coleosporium* sp. clustered into a separate clade. The three isolates (KACC 47756, KACC 44066, and KACC 44082), forming a distinct group (PP = 1.00), were closely related

with three *Lecanicillium* species, *L. muscarium* (Petch) Zare & W. Gams, *L. pissodis* Kope & I. Leal, and *L. attenuatum* Zare & W. Gams, within a strongly supported cluster (PP = 1.00).

Taxonomy

Lecanicillium uredinophilum M.J. Park, S.B. Hong & H.D. Shin, **sp. nov.** FIG. 1

MycoBank MB 814832

Differs from *Lecanicillium longisporum* by its longer phialides.

TYPE: Korea, Gangwon Province, Hongcheon, Seongdong-ri, 37°44′41″N 127°52′01″E, on uredinia of *Pucciniastrum agrimoniae* (Dietel) Tranzschel growing on *Agrimonia pilosa* Ledeb. (*Rosaceae*), 26 October 2008, collected and isolated by M.J. Park and H.D. Shin (**Holotype**, KUS-F23890; ex-type culture, KACC 44082; GenBank KM283758, KM283782, KM283806, KM283828, KM283848).

ETYMOLOGY: The epithet refers to the uredinicolous habit of this fungus.

IN VIVO: Colonies on rust sori, loose or floccose, white. Mycelium partly immersed in rust sori but mostly superficial. Phialides rather long, 15–50 × 1–3 µm, hyaline, tapering towards the apex, arising from prostrate hyphae, solitary or up to 3–4 per node; secondarily produced phialides present. Conidia 3–6 × 1.5–3 µm, hyaline, cylindric to oblong or narrowly ellipsoid, aseptate.

IN VITRO: Colonies attaining 25–30 mm diameter in 10 d on PDA, white to cream colored, velvety to cottony; margin entire; reverse cream colored. Phialides 20–60 × 1–2.5(–3) µm, produced singly or in whorls of up to 3–4(–5) on prostrate hyphae. Conidia 3–9 × 1.8–3 µm, cylindric, oblong, or ellipsoid, mostly aseptate, rarely uniseptate.

ADDITIONAL SPECIMENS EXAMINED:

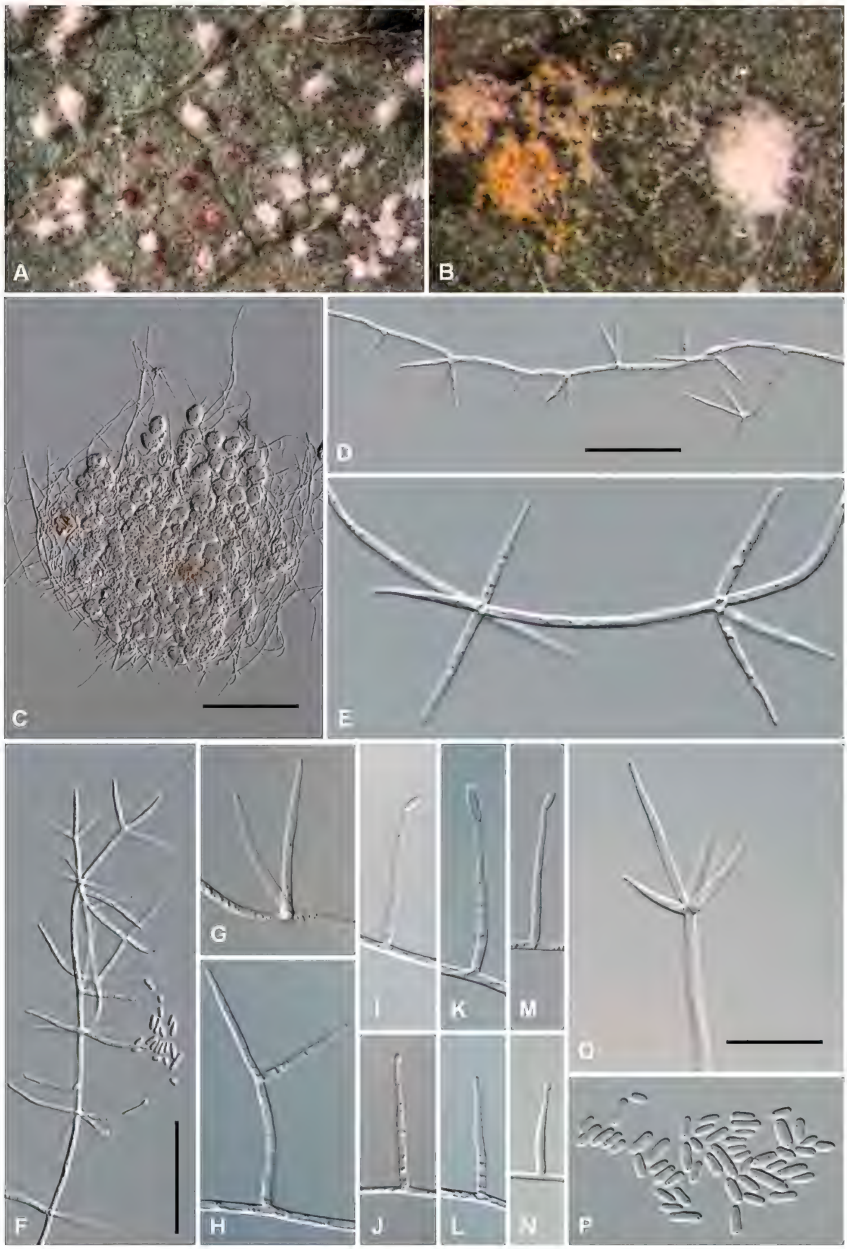
On uredinia of *Coleosporium* sp. growing on *Aster* sp. (*Asteraceae*): **KOREA, GANGWON PROVINCE**, Pyeongchang, Korea Botanic Garden, 37°42′24″N 128°36′49″E, 9 October 2008, collected and isolated by M.J. Park and H.D. Shin (KUS-F23782; culture, KACC 44066).

On uredosori of *Pucciniastrum agrimoniae* growing on *Agrimonia pilosa*: **KOREA, NORTH CHUNGCHEONG PROVINCE**, Boeun, Okhwa recreational forest, 36°35′52″N 127°41′48″E, 2 August 2009, collected and isolated by M.J. Park (KUS-F24383; culture, KACC 47756).

Discussion

Three Korean fungal isolates found as rust hyperparasites were shown to represent a new species, *Lecanicillium uredinophilum*. The morphology of

FIG. 1. *Lecanicillium uredinophilum*. A: Colonies overgrowing uredinia of *Pucciniastrum agrimoniae* on *Agrimonia pilosa*. B: Non-hyperparasitized (left) and hyperparasitized (right) uredinia. C: Rust propagules densely intertwined with hyphae of *L. uredinophilum*. D–G, O: Verticillate phialides. H: Branched phialide. I–N: Solitary phialides. P: Conidia. Scale bars: C = 100 µm, D, F = 50 µm, E, G–P = 20 µm.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L. uredinophilum resembles that of *L. longisporum* (Petch) Zare & W. Gams, which differs by its shorter phialides (20–40 µm; Zare & Gams 2001). Based on their ITS rDNA (not presented in this study) and multigene phylogenies, the three *L. uredinophilum* isolates form a well-supported distinct clade that is distant from other *Lecanicillium* taxa. Morphological and phylogenetic analyses show that the isolates represent a previously unknown species, here described as *L. uredinophilum*.

Although the main hosts of *Lecanicillium* species are known to be insects and mushrooms (Zare & Gams 2001), the present investigation indicates that rust fungi are another major ecological substrate for *Lecanicillium* species. The discovery of *Lecanicillium* species associated with rust sori suggests that more species of *Lecanicillium* and *Verticillium*-like fungi remain to be discovered as hyperparasites of rusts worldwide.

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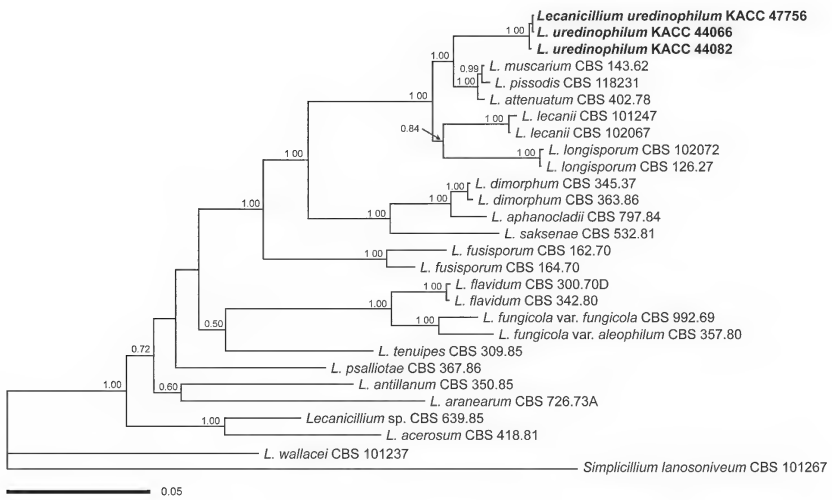
Lecanicillium uredinophilum KACC 47756
L. uredinophilum KACC 44066
L. uredinophilum KACC 44082
L. muscarium CBS 143.62
L. pissodis CBS 118231
L. attenuatum CBS 402.78
L. lecanii CBS 101247
L. lecanii CBS 102067
L. longisporum CBS 102072
L. longisporum CBS 126.27
L. dimorphum CBS 345.37
L. dimorphum CBS 363.86
L. aphanocladii CBS 797.84
L. saksenae CBS 532.81
L. fusisporum CBS 162.70
L. fusisporum CBS 164.70
L. flavidum CBS 300.70D
L. flavidum CBS 342.80
L. fungicola var. fungicola CBS 992.69
L. fungicola var. aleophilum CBS 357.80
L. tenuipes CBS 309.85
L. psalliotae CBS 367.86
L. antillanum CBS 350.85
L. aranearum CBS 726.73A
Lecanicillium sp. CBS 639.85
L. acerosum CBS 418.81
L. wallacei CBS 101237
Simplicillium lanosoniveum CBS 101267
0.05

FIG. 2. Phylogenetic relationship among taxa belonging to the genus *Lecanicillium* based on Bayesian analysis of the combined SSU, LSU, TEF1, RPB1, and RPB2 sequences. Isolates obtained from this study are in bold. The numbers at the nodes indicate the Bayesian inference posterior probability (BI PP) values. Only PP values ≥50% are shown. The number of nucleotide changes between taxa is represented by branch length and the scale bar indicates the number of nucleotide substitutions per site.

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DNA barcoding is an effective tool for differentiating *Pisolithus* species from Macedonia

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Abstract — With the aim of clarifying the number of *Pisolithus* species in Macedonia, molecular analysis was carried out on seven specimens collected in oak forests in Macedonia during 1987 and 2005. New sequences were compared with previously published sequences included in public databases. Three *Pisolithus* species are identified in Macedonia: *P. arhizus*, *P. capsulifer*, and *P. tinctorius*.

Key words — gasteromycetes, *Boletales*, ITS nrDNA, phylogeny

Introduction

Pisolithus Alb. & Schwein. is a genus widely distributed across temperate to tropical regions and forming ectomycorrhiza with many different woody plants (Marx 1977, Chambers & Cairney 1999). Phylogenetic studies conducted particularly in the last decade have pointed to the presence of several species in this genus (Anderson et al. 1998, 2001; Martin et al. 1998, 2002; Gomes et al. 2000; Díez et al. 2001) that was for a long time considered monotypic (Coker & Couch 1928, Cunningham 1942, Pilát 1958). Recently several new species have been recognized, such as *P. orientalis* Watling et al., associated with *Pinus kesiya* (Phosri et al. 2012); the new combination *P. capsulifer* (Phosri et al. 2012); *P. calongei* M.P. Martín et al., mycorrhizal with *Cistus* (Martín et al. 2013);

P. croceorrhizus P. Leonard & McMullan-Fisher associated with *Myrtaceae* (Leonard et al. 2013); a close relative of *Pisolithus* sp. 10 Martin et al. (2002); and *Pisolithus* sp. 12/13 (Leonard et al. 2013). These new analytical methods have confirmed previous hypotheses based on macroscopic and microscopic variations proposed by Bronchart et al. (1975), Calonge & Demoulin (1975), and Demoulin & Dring (1975). The only species recognized in Macedonia based on morphological features is *Pisolithus arhizus* (Karadelev et al. 2008), which is included in the Macedonian Red List of Fungi (Karadelev & Rusevska 2013). In order to determine the precise number of *Pisolithus* species in Macedonia, we decided to analyze the internal transcribed spacer region of nuclear ribosomal DNA (ITS nrDNA) obtained from several different specimens collected in Macedonia.

Materials & methods

Morphological studies

Dried samples used in this study are deposited in the Macedonian Collection of Fungi, Mycological Laboratory, Faculty of Natural Sciences and Mathematics, Sts. Cyril and Methodius University, Skopje, Macedonia (MCF). Morphological identification of the specimens has been made according to Jülich (1984), Pegler et al. (1995), Calonge (1998), Krieglsteiner (2000), Sarasini (2005), Knudsen & Vesterholt (2012). Microscopic measurements and photographs were made from tissue sections mounted in 5% KOH and examined at magnifications up to 1000× with an LW Scientific microscope. SEM was carried out after coating dried glebal samples in gold with Balzers SCD 004 sputter coater with a Hitachi S-3000N SEM. 30 spores were measured from each collection. The species distribution map was generated in ArcGIS 10.1 based on Digital Elevation Model (ASTERGDEM) (http://asterweb.jpl.nasa.gov/gdem.asp, 09. 2012).

Molecular analyses

DNA extraction, amplification, and sequencing of the ITS regions were made from glebal tissue of dry field collected basidiomes using the protocols described in Phosri et al. (2009). The ITS region, including the 5.8S rDNA the ribosomal RNA gene cluster and small flanking parts of SSU and LSU genes, was amplified by PCR with ITS1F and ITS4 primer pair (White et al. 1990, Gardes & Bruns 1993) according to the cycling protocol in Martín & Winka (2000). Five µl aliquots of PCR products were verified by gel electrophoresis in 2% Pronadisa D-1 Agarose (Lab. Conda, Spain). The amplified products were purified with QIAquick gel PCR purification kit (QAGen, Valencia, California) and sequenced by Macrogen, Inc. (South Korea). The new *Pisolithus* sequences were aligned with 99 sequences obtained from Phosri et al. (2012) and analyzed using a heuristic search in PAUP 4.0b10 (Swofford, 2003) and under a Bayesian approach (Huelsenbeck et al. 2000, Larget & Simon 1999) assuming a HKY+G

Fig. 1. Phylogenetic tree (one of 100 most parsimonious trees) obtained with a parsimony analysis under heuristic search of ITS nrDNA sequences of *Pisolithus* specimens (indicated with the MCF herbarium number) and sequences from Phosri et al. (2012), with sequences of *Suillus luteus* and *Scleroderma citrinum* as outgroup.

GU373495 Suillus luteus
FM213344 Scleroderma citrinum
C
100
AF415227 Shorea macropera, Malaysia
AF415226 Shorea macropera, Malaysia
P. aurantioscabrosus (P. species11)
AY756113 Vateria indica, India
P. indicus (P. species 13)
100
AF003915 Afzelia sp., Kenya
AF228653 Afzelia sp., Kenya
P. species 1
A I
100
FR748119 Shorea sp., Thailand
AB099921 Shorea roxburgii, Thailand
AB099920 Shorea roxburgii, Thailand
FR748120 Thailand
AB099922 Dipterocarpus alatus, Thailand
P. abditus (P. species 12)
AF003914 Eucalyptus camaldunensis, Kenya
AF004733 Eucalyptus sp., New South Wales
AF374719 Eucalyptus calophylla, Australia
99
AF004734 Eucalyptus sp., New South Wales
AF440866 Eucalyptus plantation, China
AF374665 Eucalyptus patens, Australia
P. marmoratus (P. species 2)
AF228641 Cistus ladanifer, Spain
83
FR748140-FR748142 (3 clones) Cistus sp., Spain
100
AF228642 Cistus ladanifer, Spain
AF228643 Cistus ladanifer, Spain
AF228644 Cistus ladanifer, Spain
P. calongei (P. species 3)
AF374624 Acacia sp., Australia
P. species 10
AF347700 Eucalyptus sp., Brazil
AF004735 Eucalyptus sp., New South Wales
AF142991 Eucalyptus sp., Brazil
AF228657 Eucalyptus sp., Morocco
AF140547 Eucalyptus sp., Brazil
AF374704 Eucalyptus dunni, Brazil
U62666 Eucalyptus citriodora, Brazil
56
66
AF374661 Eucalyptus camaldunensis, Australia
AF374708 Eucalyptus sp./Acacia sp., Australia
AF004732 Eucalyptus sp., New South Wales
P. microcarpus (P. species 9)
AF270786 Eucalyptus sp., Australia
AF374622 Acacia holosericea, Senegal
FR748122 Eucalyptus camaldunensis,Thailand
FR748123 Eucalyptus camaldunensis,Thailand
AF228656 Eucalyptus sp., Spain
87
AF228655 Eucalyptus sp., Morocco
AF228654 Eucalyptus sp., Morocco
AB099918 Thailand
FR748121 Eucalyptus camaldunensis,Thailand
AB099909 Eucalyptus camaldunensis,Thailand
B 99
AF416589 Eucalyptus tereticornis, India
AF440868 Eucalyptus sp., China
AF374675 Eucalyptus sp., Queensland
AF004736 unknown potential host, New South Wales
AF004737unknown potential host, New South Wales
FR748124 Eucalyptus camaldunensis, Thailand
AJ629887 Thailand
FR748125 Eucalyptus sp., Hawaii, USA
FR748127 Melaleuca sp., Thailand
AB099911 Thailand
AF374638 Acacia sp./Mangium sp., Malaysia
FR748126 Eucalyptus sp., Australia
P. albus complex
(P. species 7)
AF374646 Eucalyptus globulus, Australia
P. species 10
AF440867 Eucalyptus plantation, China
P. microcarpus (P. species 9)
AF003916 Pinus caribea, Kenya
AF228645 Quercus ilex/Q. coccifera, Spain
AF228646 Quercus ilex/Cistus ladanifer, Spain
FR748138 Pinus sp., Spain
AF143234 Unknown potentital host, France
58
AF143233 Pinus sp., USA
05MCF5506
FR748139 Quercus sp., USA
AF228647 Pinus caribea, Kenya
P. tinctorius (P. species 6)
93
AF374625 Pinus kesiya, Thailand
AF374679 Eucalyptus sp., China
AF374711 Pinus sp./Eucalyptus sp., China
AB099919 Pisolithus sp., Thailand
FR748149 Pinus kesiya, Thailand
54
FR748147Pinus kesiya, Thailand
FR748146 Pinus kesiya, Thailand
AB099845 Pisolithus sp., Thailand
FR748145 Pinus kesiya, Thailand
FR748148 Pinus kesiya, Thailand
FR748143 Pinus kesiya, Thailand
FR748144 Pinus kesiya, Thailand
P. orientalis (P. species 5)
A II
FM213365 Pinus sp., Spain
AF228648 Quercus ilex/Q. coccifera, Spain
FR748128 (clone a),FR748131(clone d) Will Wood, Sardinia, Italy
02MCF9560
05MCF5324
87MCF7741
FR748130 (clone c) Will Wood, Sardinia, Italy
98MCF4331
FR748129 (clone b) Will Wood, Sardinia, Italy
AF228649 Quercus ilex, Spain
AF228651 Quercus ilex, Spain
54
AF228650 Pinus halepensis/Quercus coccifera, Spain
AF228652 Pinus halepensis, Spain
FR748132 (clone f) Will Wood, Sardinia, Italy
FR748133 Pinus sp., Spain
AF374627 Pinus sp. South Africa
P. arhizus (P. species 4)
AF374629 Pinus pumila/Betula ermanii, Japan
UDB001206 Pinus forest, Sweden
78
03MCF3428
FR748134 unknown potential host, England
02MCF3355
FR748135 unknown potential host, England
FR748138 mixed forest, France
FR748136 Pinus sylvestris/Pinus contorta, England
P. capsulifer (P. species 14)
– 5 changes

model with MRBAYES 3.0 (Huelsenbeck & Ronquist 2001), as described in Phosri et al. (2012). The phylogenetic tree was visualized in Treeview (Page 1996) and edited in Adobe Illustrator CS3. The names of clades and subclades follow Phosri et al. (2009).

Results

Genomic DNA concentration ranged from 1.5 to 15 ng/µl. The ITS region was successfully amplified and sequenced from 7 collections of dried *Pisolithus* basidiomes using ITS1F/ITS4 primer pair. After purification of the amplified product, the DNA concentration was greater than 20 ng/µl, and sequenced directly. The seven new sequences obtained in this study have been submitted to the international database (EMBL).

The phylogenetic tree (Fig. 1) obtained under heuristic search has similar topology with the 50% majority-rule tree of the Bayesian analysis (data not shown). New sequences are well grouped in linage AII (Phosri et al. 2012), but in three main clades, each corresponding to one *Pisolithus* species.

One sample grouped in the *P. tinctorius* clade. Four *Pisolithus* records that were placed in the *P. arhizus* clade were recorded from three different localities: Pogana (kermes oak shrubland), Galichica Mountain (Macedonian oak forest), and Skopska Crna Gora Mountain (oak–hornbeam forest). Two *Pisolithus* collections, both originating from Bistra Mountain, were placed in the *P. capsulifer* clade.

Pisolithus tinctorius (Pers.) Coker & Couch,
Gasteromycetes East. U.S.: 170. 1928. Fig. 2

Basidiomes pyriform with almost ellipsoid head, slightly flattened at the apex, 55–60 × 40–50 mm and a pseudostipe, 40–50 × 20–25 mm, narrowing toward the base, ≤10 mm; peridium yellowish olivaceous, very thin (<1 mm), showing pseudoperidioles as black ellipsoid patches.

Basidiospores globose, 8.4–11.6 µm, in average 9.9 µm (including spines); spines 1.2–2.3 µm (average = 1.6 µm) long, more or less isolated.

Specimen examined: **MACEDONIA, Skopska Crna Gora Mountain**, 800–900 m a.s.l., in meadow close to deciduous forest (*Quercus* spp., *Carpinus orientalis*, and planted *Castanea sativa*), 9-Oct-2010, leg. K. Rusevska (05MCF5506, LK024184).

Pisolithus arhizus (Scop.) Rauschert, Z. Pilzk. 25: 51. 1959. Fig. 3

Basidiomes with subglobose to ellipsoid head, 30–60 × (40–)55–90 mm tapering into irregular pseudostipe ≤95 mm × (20–)25–40(–50) mm, with narrow base ≤10 mm diam; peridium pale ochre to brown black.

Basidiospores globose, 8.4–11.6 µm, in average 9.9 µm (including spines); spines 1.2–2.3 µm (average = 1.2 µm) long, isolated but very compact, in parts forming small pyramids.

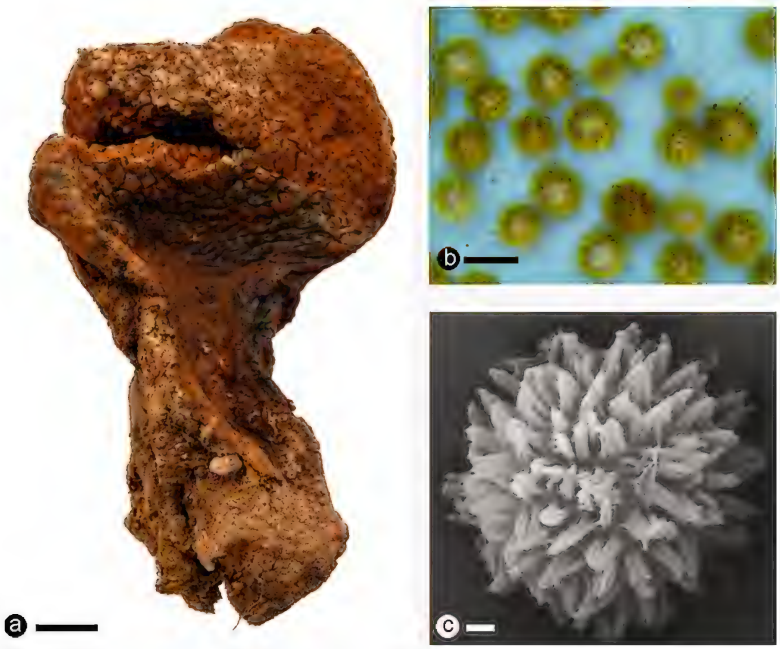

Fig. 2. *Pisolithus tinctorius* (05MCF5506). a. basidioma; b. spores (LM); c. spores (SEM). Scale bars: a = 1 cm; b = 10 μm; c = 1 μm.

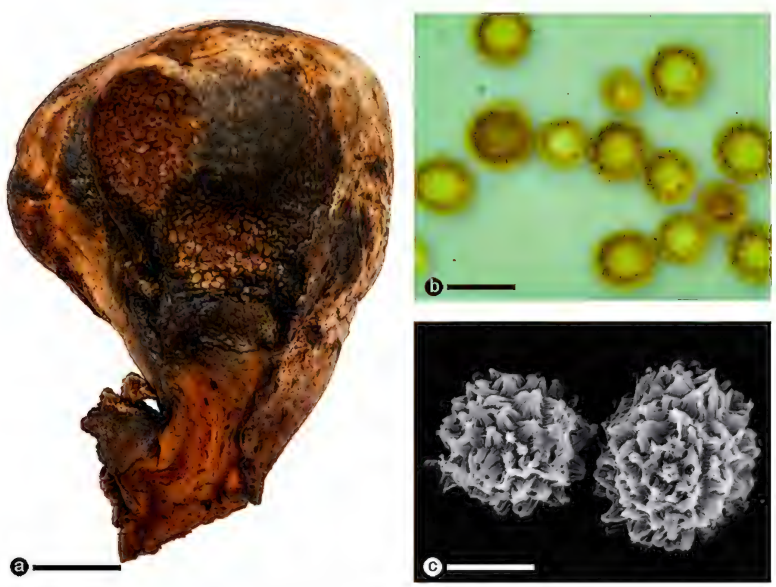

Fig. 3. *Pisolithus arhizus* (05MCF5324). a. basidioma; b. spores (LM); c. spores (SEM). Scale bars: a = 1 cm; b = 10 μm; c = 5 μm.

Specimens examined: **MACEDONIA**, **Galichica National Park**, between Oteshevo and Kale, 900–1000 m a.s.l., Macedonian oak forest, 8-Oct-1988, leg. M. Karadelev (02MCF9560, LK024180); Gradishte, at roadsides, 800 m a.s.l., grassy place in Macedonian oak forest, 10-Nov-2005, leg. K. Rusevska (05MCF5324, LK024181); **Pogana**, 300 m a.s.l., meadow in *Quercus coccifera* forest, 28-Oct-1987, leg. M. Karadelev (87MCF7741, LK024178); **Skopska Crna Gora**, above Chucher-Sandevo vill., 600 m a.s.l., degraded oak forest (*Querco-Carpinetum orientalis*), 6-Oct-1998, leg. M. Karadelev (98MCF4331, LK024179).

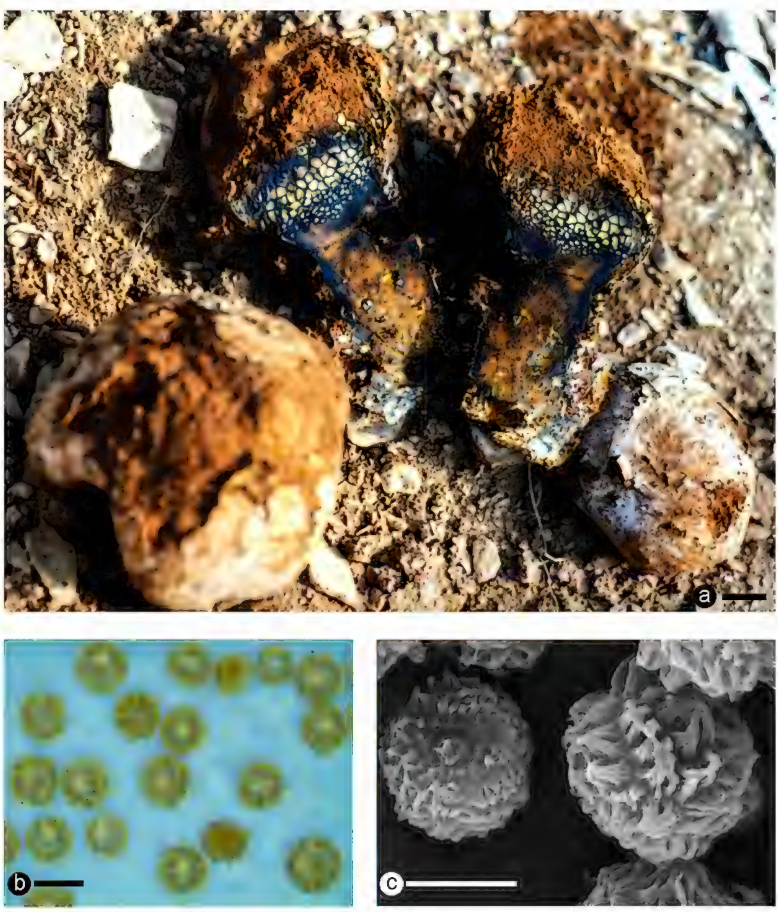

Fig. 4. *Pisolithus capsulifer* (03MCF3428). a. basidioma; b. spores (LM); c. spores (SEM). Scale bars: a = 1 cm; b = 10 µm; c = 5 µm.

Pisolithus capsulifer (Sowerby) Watling, Phosri & M.P. Martín, Mycotaxon 120: 202. 2012. Fig. 4

Basidiomes with globose to subglobose head, 25–35 × 45 mm, pseudostipe 40 mm long, 20–22 mm wide, and incorporating soil particles; peridium pale

ocher to black; PSEUDOPERIDIOLES dark yellow when cut, becoming darker and finally black on drying.

BASIDIOSPORES globose, 6.8–11.0 µm, in average 9.3 µm (including spines); spines 1.1–2.2 µm (average = 1.6 µm) long, mainly united in conical structures.

SPECIMENS EXAMINED: **MACEDONIA, BISTRA MOUNTAIN**, above Tresonche vill., 1100 m a.s.l., open place in deciduous forest, on cinnamon soil, 1100 m a.s.l., 3-Aug-2002, leg. K. Rusevska (02MCF3355, LK024182); 12-Jul-2003, leg. K. Rusevska (03MCF3428, LK024183).

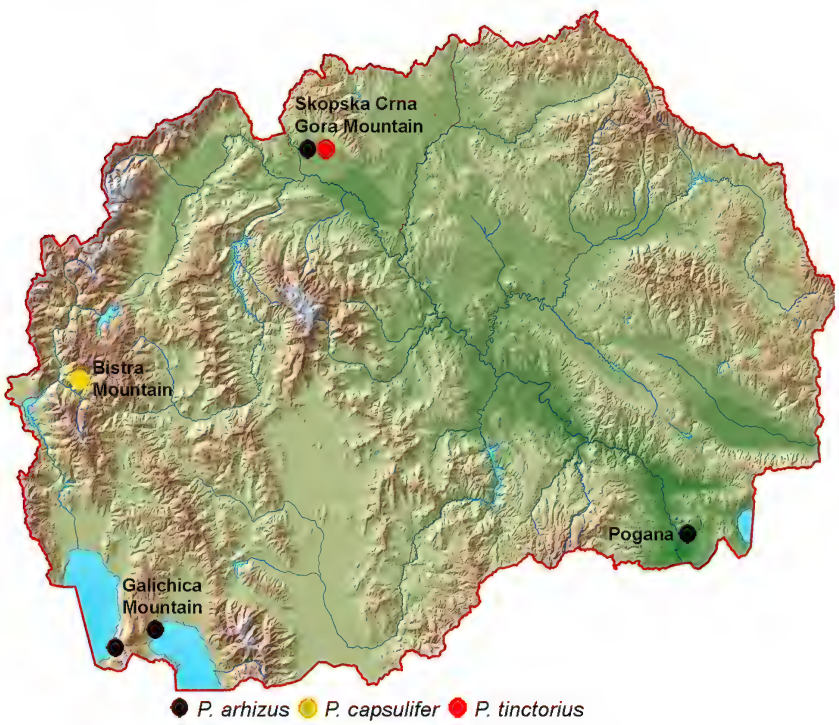

FIG. 5. Distribution map of *Pisolithus* collections from Macedonia analyzed in this study.

Discussion

The analyzed *Pisolithus* samples were obtained from four different localities in Macedonia: Pogana, and mountains Bistra, Galichica, and Skopska Crna Gora (FIG. 5).

Bistra Mountain is situated in western Macedonia. *Pisolithus capsulifer*, a new species for the mycobiota of Macedonia, was found near Tresonche village. The samples were collected at approximately 1000 m on cinnamon soil in an open area with common juniper shrubs. The site is close to a xerophytic and

thermophilic community of pubescent oak and hop hornbeam as dominant trees; *Acer monspessulanum* and *Fraxinus ornus* are also present. *Pisolithus capsulifer* is known from a few sites in three European countries (Sweden, France, and England) and Japan (Phosri et al. 2012).

Pogana, which lies in southern Macedonia, is one of three *P. arhizus* localities. The dominant forest species, evergreen shrub kermes oak, forms a well-developed forest community (*Coccifero-Carpinetum orientalis*) together with *Quercus pubescens*, as well as *Phillyrea media,* the dominant evergreen shrub. Several other plant species also present are *Pistacia terebinthus*, *Fraxinus ornus*, *Coronilla emeroides*, *Colutea arborescens*, *Crataegus heldreichii*, *Lonicera etrusca*, and *Cistus villosus.*

Galichica Mountain is situated between two lakes in southwestern Macedonia. *Pisolithus arhizus* is known from two mountain sites (one on the Lake Ohrid side and the other on the Lake Prespa side) between 800 and 1000 m altitude. The collections were made in Macedonian oak forest with *Quercus trojana* dominant and *Q. cerris, Q. pubescens, Carpinus orientalis,* and *Fraxinus ornus* also present.

The mountain Skopska Crna Gora lies in northern Macedonia. Two *Pisolithus* species were found on different sites in oak-hornbeam forests. *Pisolithus arhizus* was collected at 600 m from a degraded forest, while *P. tinctorius* was recorded at 800 m in a well-developed association mixed with planted sweet chestnut trees.

Using ITS barcodes (see Schoch et al. 2012) to analyze fungi has greatly clarified the *Pisolithus* complex in Macedonia. Our study confirms the presence of three *Pisolithus* species in the country: *P. arhizus, P. capsulifer,* and *P. tinctorius*, all growing near oak trees in deciduous forests. Thus far, *P. capsulifer* appears specific to a single locality and substrate (terra rossa on silicate ground). *Pisolithus arhizus,* as noted, is already included in the National Red List of Fungi. The two new species for Macedonia, *P. capsulifer* and *P. tinctorius*, are also rare and should be protected.

As *P. tinctorius* and *P. arhizus* have the same spore size and *P. arhizus* and *P. capsulifer* have a similar peridium colors, it is almost impossible to separate these species morphologically. In the face of such similar microscopic and macroscopic characters, we do not propose a key here.

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Five new species of *Hymenoscyphus* (*Helotiaceae, Ascomycota*) with notes on the phylogeny of the genus

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Abstract — Five new species of *Hymenoscyphus*—*H. aurantiacus*, *H. macrodiscus*, *H. qinghaiensis*, *H. tetrasporus*, and *H. yui*—are recognized by morphological characters and DNA sequence data. Detailed descriptions, illustrations, and discussions concerning morphologically similar and phylogenetically closely related species are provided for each species. The phylogeny of 39 *Hymenoscyphus* species was explored based on the individual internal transcribed spacer of nrDNA, D1/D2 domain of 28S nrDNA, and partial β-tubulin gene sequences, as well as the combined dataset. The sampled species clustered as a highly supported group in the phylogenetic trees with six main clades formed. Correlations between the morphological and phylogenetic features are discussed.

Key words — China, *Leotiomycetes*, taxonomy

Introduction

The genus *Hymenoscyphus* was established by Gray (1821) and lectotypified with *H. fructigenus* (Bull.) Gray (Dennis 1964). It is one of the largest genera of *Helotiaceae* and has a cosmopolitan distribution (Kirk et al. 2008). About 155 species were known in the genus according to Kirk et al. (2008) with a few more species added subsequently (Han & Shin 2008; Queloz et al. 2011; Baral & Bemmann 2013; Zheng & Zhuang 2013a,c, 2014; Gross & Han 2015; Gross et al. 2015).

Species of *Hymenoscyphus* are mainly saprotrophic on plant materials such as wood, twigs, fruits, leaves and herbaceous stems, producing small, stipitate to sessile, white- to yellow-colored apothecia and having an ectal excipulum composed of non-gelatinized textura prismatica to textura angularis parallel or at a low angle to the receptacle surface and fusoid, ellipsoid, to scutuloid hyaline ascospores. Morphological characteristics used to identify *Hymenoscyphus*

species include apothecial shape, size, and color; excipular structure; the ascal apical apparatus, basal structure, shape, and size; and shape, size, guttulation, septation, and presence of cilia of ascospores. Habitat is another useful feature for species recognition. Sequence data of the internal transcribed spacer of nuclear ribosomal DNA (ITS) have been introduced to aid identification (Zhang & Zhuang 2004, Baral et al. 2006, Han & Shin 2008, Johnston & Park 2013, Zheng & Zhuang 2013a), with multilocus phylogenetic approaches further applied to establish a clear species concept of the *H. albidus* complex (Queloz et al. 2011, Zheng & Zhuang 2014, Gross & Han 2015, Gross et al. 2015).

A total of 42 *Hymenoscyphus* species have been reported from 28 provinces of China (Teng 1963; Tai 1979; Korf & Zhuang 1985, 1989; Zhuang 1995, 1996, 1999; Wu et al. 1996; Zhuang & Wang 1998; Yu et al. 2000; Zhang & Zhuang 2002a,b, 2004; Zheng & Zhuang 2011, 2013a,b,c, 2014), of which 12 were originally described from China (Zhuang 1996; Zhang & Zhuang 2002a,b, 2004; Zheng & Zhuang 2013a,c, 2014; Liu & Zhuang 2015). In this work, five new species are established based on morphological and molecular data.

Materials & methods

Morphological study

Specimens examined were collected from Hubei, Qinghai, Xinjiang, and Yunnan provinces and deposited in Herbarium Mycologicum, Academiae Sinicae, Beijing, China (HMAS). Colors of fresh apothecia were recorded in field notes. Dry apothecia were rehydrated with distilled water and sectioned longitudinally at a thickness of 8–15 µm using a Yidi YD-1508A freezing microtome (Jinhua, China). Measurements were taken from the longitudinal sections and from squash mounts in lactophenol cotton blue solution using an Olympus BH-2 microscope (Tokyo, Japan). Iodine reactions of ascus apparatuses were tested in Melzer's reagent and Lugol´s solution with or without pretreatment by 3% KOH (Baral 2009). Features of ascus apical ring were recorded according to Baral (1986, 1987). Photographs were taken using a Canon G5 digital camera (Tokyo, Japan) connected to a Zeiss Axioskop 2 Plus microscope (Göttingen, Germany) for anatomical structure (lactophenol cotton blue as mounting medium except for otherwise mentioned) and to a Zeiss Stemi 2000C stereomicroscope (Göttingen, Germany) for gross morphology.

DNA extraction, PCR amplification, and sequencing

DNA was extracted from dry apothecia using the CTAB procedure (White et al. 1990) with some modifications. Primer pairs used for amplification and sequencing included ITS1/ITS4 or ITS5/ITS4 (White et al. 1990) for ITS, LR0R (Moncalvo et al. 1995) and LR5 (Vilgalys & Hester 1990) for D1/D2 domain of 28S nrDNA (LSU) and Bt3-LM/Bt10-LM (Myllys et al. 2001) for partial β-tubulin gene (BTU).

PCR amplifications had a final volume of 25 µl, containing 2.5 µl 10× PCR buffer (including $MgCl_2$), 0.5 µl dNTP (10mM each), 1.25 µl of each primer (10 mM),

TABLE 1. Sequences of *Hyaloscypha* (*Hya.*) and *Hymenoscyphus* (*H.*) used in the phylogenetic analyses, with voucher specimen information and GenBank accession numbers

SPECIES	SPECIMEN/ STRAIN NO.	ITS	LSU	BTU
Hya. aureliella	M234	EU940228	EU940152	FJ477050
Hya. fuckelii	M233	EU940230	EU940154	FJ477052
H. albidoides H.D. Zheng & W.Y. Zhuang	HMAS 264140	KF188722	KJ472226	KF597265
	HMAS 264142	KF188723	KJ472227	KF597267
H. albidus (Gillet) W. Phillips	Lav_01	GU586884	—	—
	90812.1	GU586893	—	—
H. aurantiacus	HMAS 264143	KJ472289	KJ472228	KJ472259
	HMAS 264144	KJ472288	KJ472229	KJ472260
H. berggrenii (Cooke & W. Phillips) Kuntze	ICMP 19614	KC164645	KC164640	—
	ICMP 19615	KC164647	—	—
H. brevicellulus H.D. Zheng & W.Y. Zhuang	HMAS 264018	JX977162	KJ472231	KJ472262
	HMAS 264016	JX977155	KJ472230	KJ472261
H. calyculus	HMAS 264145	KJ472290	KJ472232	KJ472263
	HMAS 264146	KJ472291	KJ472233	KJ472264
H. caudatus	HMAS 82057	AY348576	KJ472234	KJ472265
	HMAS 82060	AY348577	KJ472235	KJ472266
H. crataegi Baral & R. Galán	F156966	DQ431177	—	—
H. dehlii M.P. Sharma	HMAS 264160	KC416307	—	—
H. epiphyllus (Pers.) Rehm ex Kauffman	HMAS 82075	AY348580	KJ472236	KJ472267
	HMAS 82076	AY348581	KJ472237	KJ472268
H. fraxineus (T. Kowalski) Baral et al.	HMAS 264174	KF188724	KJ472248	KF597268
	HMAS 266580	KF188726	KJ472249	KF597269
H. fructigenus	Oth_01	GU586904	—	—
	HMAS 75893	JX977144	—	—
	CBS650.92	GU586933	—	—
	ASM 1061	—	JN673046	—
	M159	—	EU940157	FJ477056
H. fucatus (W. Phillips) Baral & Hengstm.	HMAS 264028	JX977147	—	—
	HMAS 264029	JX977148	—	—
H. ginkgonis J.G. Han & H.D. Shin	KUS-F51352	EU096525	—	—
	KUS-F51854	EU219982	—	—
H. globus W.Y. Zhuang & Yan H. Zhang	HMAS 82107	AY348593	KJ472238	KJ472269
H. haasticus P.R. Johnst.	ICMP 19598	KC164648	—	—
	ICMP 19603	KC164644	KC164639	—
H. himalayensis (K.S. Thind & H. Singh) K.S. Thind & M.P. Sharma	HMAS 188545	KJ472292	—	—
H. hyaloexcipulus	HMAS 188542	JX977145	—	—

TABLE 1, concluded on p. 1020

TABLE 1, concluded

SPECIES	SPECIMEN/ STRAIN NO.	ITS	LSU	BTU
H. immutabilis	HMAS 71809	AY348584	KJ472239	KJ472270
	F155012	DQ431175	—	—
H. jinggangensis Yan H. Zhang & W.Y. Zhuang	HMAS 82036	KJ472293	KJ472240	KJ472271
	HMAS 188548	KJ472294	KJ472241	—
	HMAS 264156	KJ472295	—	KJ472272
H. kiko P.R. Johnst.	ICMP 19613	KC164656	—	—
	ICMP 19597	KC164661	KC164642	—
H. lasiopodius (Pat.) Dennis	HMAS 71820	AY348585	KJ472242	KJ472273
	HMAS 75878	AY348587	KJ472243	KJ472274
H. macrodiscus	HMAS 264158	KJ472296	—	—
H. macroguttatus	HB7034	DQ431179	—	—
	HMAS 264159	KC416306	KJ472244	KJ472275
H. microcaudatus H.D. Zheng & W.Y. Zhuang	HMAS 264020	JX977156	KJ472245	KJ472276
H. microserotinus (W.Y. Zhuang) W.Y. Zhuang	HMAS 68520	DQ986481	—	—
	HMAS 264030	JX977150	KJ472246	KJ472277
	HMAS 264031	JX977151	KJ472247	KJ472278
H. cf. *monticola*	HMAS 188562	KJ472301	—	—
H. ohakune P.R. Johnst.	ICMP 19601	KC164649	—	—
	ICMP 19607	KC164650	—	—
H. qinghaiensis	HMAS 264175	KJ472297	—	—
H. scutula (Pers.) W. Phillips	HMAS 264178	KC416308	—	KJ472279
	HMAS 264180	KC416309	KJ472250	—
H. scutuloides	HMAS 82092	AY348589	KJ472251	KJ472280
	HMAS 82093	AY348590	KJ472252	KJ472281
H. serotinus	F093261	DQ431168	—	—
	F156526	DQ431178	—	—
H. sinicus	HMAS 71818	KJ472298	—	KJ472282
	HMAS 264193	KJ472299	KJ472253	KJ472283
	HMAS 264194	KJ472300	KJ472254	-
H. subpallescens Dennis	HMAS 264022	JX977154	KJ472255	KJ472284
	HMAS 264026	JX977160	KJ472256	KJ472285
H. subsymmetricus H.D. Zheng & W.Y. Zhuang	HMAS 264021	JX977153	KJ472257	KJ472286
H. tamaricis R. Galán et al.	br020	DQ431167	—	—
	F110125	—	FJ005156	—
H. tetrasporus	HMAS 266592	KJ472302	—	—
H. waikaia P.R. Johnst.	PDD 102886	KC164667	—	—
	ICMP 19599	KC164646	KC164641	—
H. yui	HMAS 266594	KJ472304	—	—
	HMAS 266595	KJ472303	KJ472258	KJ472287

2.5 units of Taq DNA polymerase (HT-biotech Co., Ltd., Beijing, China), and 2–3 µl DNA template. PCR reactions were carried out in an Applied Biosystems 2720 thermocycler (Foster City, CA, USA) under the following conditions: an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30–60 s, annealing at 50–53 °C for 30–60 s, and extension 1 min at 72 °C, and by a final extension at 72 °C for 10 min.

PCR products were purified using the AxyPrep™ 96-well DNA Gel Extraction Kit (Corning Life Sciences – Axygen Biotechnology Co., Ltd., Hangzhou, China) and sequenced on an ABI 3730 XL DNA Sequencer (Applied Biosciences, Foster City, CA, USA) with the same primer pairs used for PCR amplification at Beijing Tianyi Huiyuan Bioscience and Technology Inc., China.

Sequence assembly, alignment and phylogenetic analyses

Forward and reverse sequences generated in this study were assembled using BioEdit 7.0.5.3 (Hall 1999) or SeqMan (DNASTAR, Lasergene 7.1.0) to obtain a consensus sequence. Sequences used in the phylogenetic analyses were either generated by this study or retrieved from GenBank (Table 1). *Hyaloscypha aureliella* (Nyl.) Huhtinen and *Hyaloscypha fuckelii* Nannf. were used as outgroup taxa. Sequences were aligned with either BioEdit 7.0.5.3 or Muscle 3.8.31 (Edgar 2004), manually edited when needed, and converted to nexus files in ClustalX 1.83 (Thompson et al. 1997).

Phylogenetic analyses were based on individual ITS, LSU and BTU sequences as well as combined dataset. All characters were treated as unordered and equally weighted, and gaps were treated as missing data. Prior to combined analyses, partition homogeneity tests (PHT) were performed in PAUP* 4.0b10 (Swofford 2003) with 100 replicates to confirm the combination of different gene sequences for phylogenetic analyses. PAUP*4.0b10 for Windows (Swofford 2003) was used to conduct neighbor-joining (NJ) and maximum parsimony (MP) analyses. NJ analysis was performed using default settings. MP analysis was done with the heuristic search option using max trees set to 1000 and auto-increased by 100, TBR branch swapping. Tree scores, including tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for all the trees generated. The topological confidence of the resulting trees was tested with bootstrap analysis using 1000 replications, each with 10 replicates of random addition of taxa in both NJ and MP analyses.

Bayesian inference (BI) analysis was performed using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001) under the best-fit evolutionary model estimated by MrModeltest 2.3 with the Akaike information criterion (Nylander 2004). Two parallel runs of four simultaneous chains of Markov Chain Monte Carlo were performed starting from random trees for 2,000,000 generations with the trees sampled every 100 generations (resulting in 20,000 total trees). The first quarter of sampled trees were discarded as the burn-in phase of the analyses, and the remaining trees were used for calculating posterior probabilities (PP) in the majority rule consensus tree. The trees were viewed in TreeView 1.6.6 (Page 1996).

Results

ITS, LSU, and BTU sequences of *Hymenoscyphus* were analyzed separately as well as concatenated. The P value of PHT is 0.06 for the combined ITS, LSU,

and BTU matrix, which indicates no significant incongruence among the three markers. For Bayesian analysis, GTR+I+G was selected by MrModeltest as the best-fit model for all the four datasets. The characteristics of each sequence dataset are summarized in Table 2.

Table 2. Sequence characteristics of ITS, LSU, BTU, and combined datasets.

Parameter	ITS	LSU	BTU	Combined dataset
Number of *Hymenoscyphus* sequences (newly generated)	70 (18)	40 (33)	34 (29)	30 (30)
Number of *Hymenoscyphus* species / represented by holotype (newly generated / new species + known species)	39 / 19 (9 / 5+4)	26 / 8 (20 / 2+18)	21 / 9 (20 / 2+18)	19 / 8 (19 / 2+17)
Total nucleotides	593	844	804	2193
Constant characters	345	720	512	1592
Variable but parsimony uninformative characters	44	16	26	87
Parsimony informative characters	204	108	266	514
Tree length (TL)	966	250	950	1622
Consistency index (CI)	0.424	0.620	0.457	0.533
Retention index (RI)	0.788	0.846	0.685	0.740
Rescaled consistency index (RC)	0.334	0.524	0.313	0.394
Homoplasy index (HI)	0.576	0.380	0.543	0.467
Best-fit model	GTR+I+G	GTR+I+G	GTR+I+G	GTR+I+G

The NJ, MP, and BI analyses produced similar tree topologies except for minor differences in the arrangement of a few terminal branches. The phylograms inferred from NJ analysis of each dataset are shown in Figs 1–3 and statistical supporting values higher than 50% or 0.50 are shown at the nodes. In the phylogenetic trees inferred from all datasets, the sampled species of *Hymenoscyphus* formed a strongly supported group.

The ITS alignment included 70 sequences representing 39 *Hymenoscyphus* species with 593 nucleotides. The ITS barcodes recognized all five putative new taxa that appeared as independent lineages, which give strong support to the morphological observations. In the ITS tree (Fig. 1), the genus were divided into six main clades (A, B, C, D, E, and F) with high statistic supports and a few scattered species. The LSU dataset consisted of 40 samples from 26 species (including two new species) with 844 characters. The LSU tree (Fig. 2A) discriminated the clades A, D, E, and F, while Clade B split into two parts, one together with Clade A and the other with species of Clade C. The BTU matrix comprised 34 sequences from 21 species (including two new species)

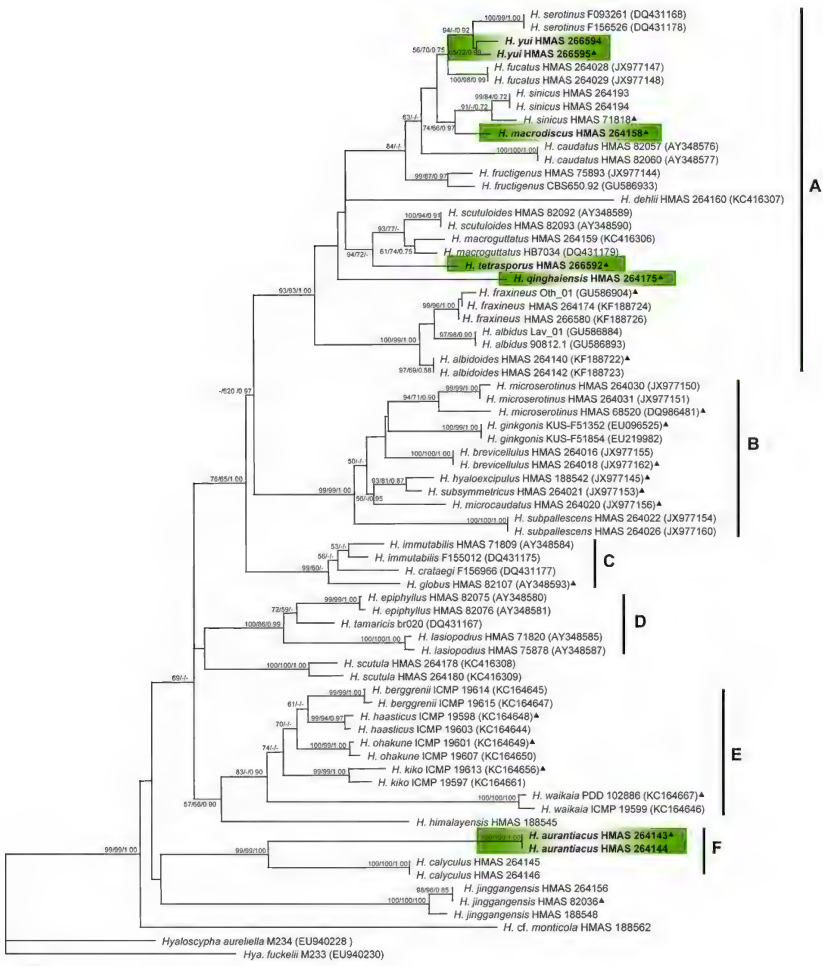
H. serotinus F093261 (DQ431168)
H. serotinus F156526 (DQ431178)
H. yui HMAS 266594
H.yui HMAS 266595▲
H. fucatus HMAS 264028 (JX977147)
H. fucatus HMAS 264029 (JX977148)
H. sinicus HMAS 264193
H. sinicus HMAS 264194
H. sinicus HMAS 71818▲
H. macrodiscus HMAS 264158▲
H. caudatus HMAS 82057 (AY348576)
H. caudatus HMAS 82060 (AY348577)
H. fructigenus HMAS 75893 (JX977144)
H. fructigenus CBS650.92 (GU586933)
H. dehlii HMAS 264160 (KC416307)
H. scutuloides HMAS 82092 (AY348589)
H. scutuloides HMAS 82093 (AY348590)
H. macroguttatus HMAS 264159 (KC416306)
H. macroguttatus HB7034 (DQ431179)
H. tetrasporus HMAS 266592▲
H. qinghaiensis HMAS 264175▲
H. fraxineus Oth_01 (GU586904)▲
H. fraxineus HMAS 264174 (KF188724)
H. fraxineus HMAS 266580 (KF188726)
H. albidus Lav_01 (GU586884)
H. albidus 90812.1 (GU586893)
H. albidoides HMAS 264140 (KF188722)▲
H. albidoides HMAS 264142 (KF188723)
H. microserotinus HMAS 264030 (JX977150)
H. microserotinus HMAS 264031 (JX977151)
H. microserotinus HMAS 68520 (DQ986481)▲
H. ginkgonis KUS-F51352 (EU096525)▲
H. ginkgonis KUS-F51854 (EU219982)
H. brevicellulus HMAS 264016 (JX977155)
H. brevicellulus HMAS 264018 (JX977162)▲
H. hyaloexcipulus HMAS 188542 (JX977145)▲
H. subsymmetricus HMAS 264021 (JX977153)▲
H. microcaudatus HMAS 264020 (JX977156)▲
H. subpallescens HMAS 264022 (JX977154)
H. subpallescens HMAS 264026 (JX977160)
H. immutabilis HMAS 71809 (AY348584)
H. immutabilis F155012 (DQ431175)
H. crataegi F156966 (DQ431177)
H. globus HMAS 82107 (AY348593)▲
H. epiphyllus HMAS 82075 (AY348580)
H. epiphyllus HMAS 82076 (AY348581)
H. tamaricis br020 (DQ431167)
H. lasiopodius HMAS 71820 (AY348585)
H. lasiopodius HMAS 75878 (AY348587)
H. scutula HMAS 264178 (KC416308)
H. scutula HMAS 264180 (KC416309)
H. berggrenii ICMP 19614 (KC164645)
H. berggrenii ICMP 19615 (KC164647)
H. haasiicus ICMP 19598 (KC164648)▲
H. haasiicus ICMP 19603 (KC164644)
H. ohakune ICMP 19601 (KC164649)▲
H. ohakune ICMP 19607 (KC164650)
H. kiko ICMP 19613 (KC164656)▲
H. kiko ICMP 19597 (KC164661)
H. waikaia PDD 102886 (KC164667)▲
H. waikaia ICMP 19599 (KC164646)
H. himalayensis HMAS 188545
H. aurantiacus HMAS 264143▲
H. aurantiacus HMAS 264144
H. calyculus HMAS 264145
H. calyculus HMAS 264146
H. jinggangensis HMAS 264156
H. jinggangensis HMAS 82036▲
H. jinggangensis HMAS 188548
H. cf. monticola HMAS 188562
Hyaloscypha aureliella M234 (EU940228)
Hya. fuckelii M233 (EU940230)
0.01
A
B
C
D
E
F

FIG. 1. Neighbor-joining tree of selected *Hymenoscyphus* species inferred from ITS sequences. Bootstrap values of NJ (left) and MP (middle) > 50%, and Bayesian posterior probabilities values (right) >0.50 are indicated at the nodes. ▲ indicates an ex-holotype sequence.

containing 804 positions. The clades A, B, C, D, and F were well reconstructed in the tree inferred from BTU with Clade E missing due to unavailability of sequence data (FIG. 2B). The combined three-marker alignment consisted of 30 samples representing 19 species and 2,193 characters including two new species. In the tree generated from the combined dataset (FIG. 3), the clades A, B, C, D, and F were well recognized.

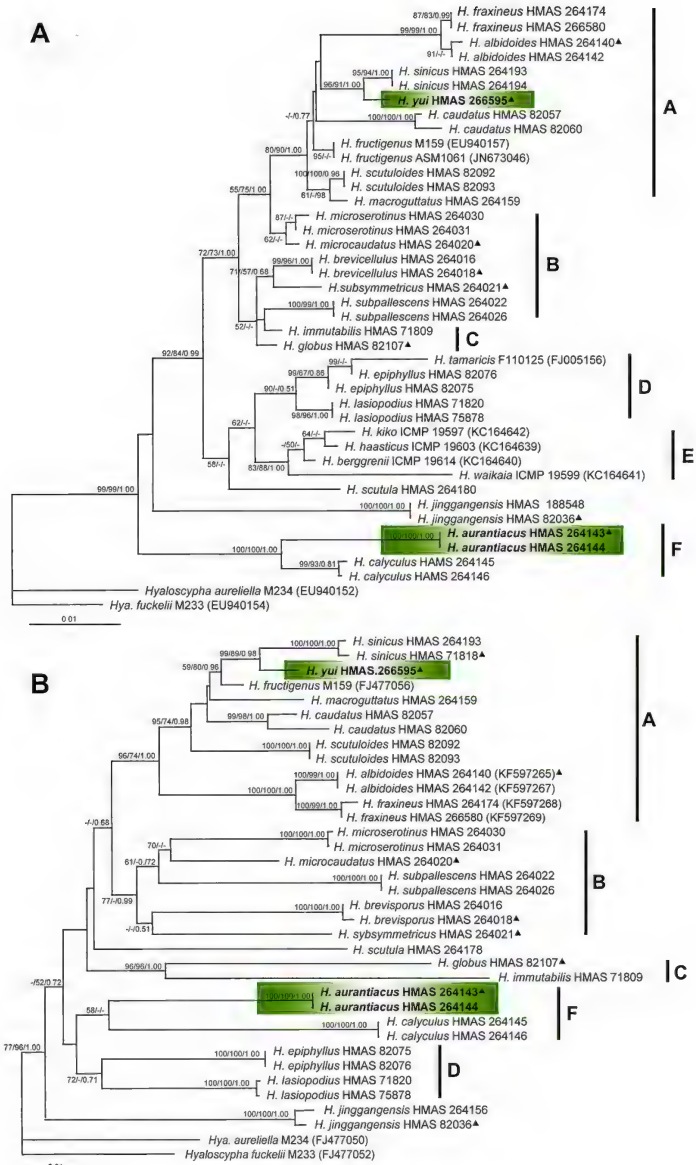

FIG. 2. Neighbor-joining trees of selected *Hymenoscyphus* species inferred from LSU (A) and BTU (B) sequences. Bootstrap values of NJ (left) and MP (middle) > 50%, and Bayesian posterior probabilities values (right) >0.50 are indicated at the nodes. ▲ indicates an ex-holotype sequence.

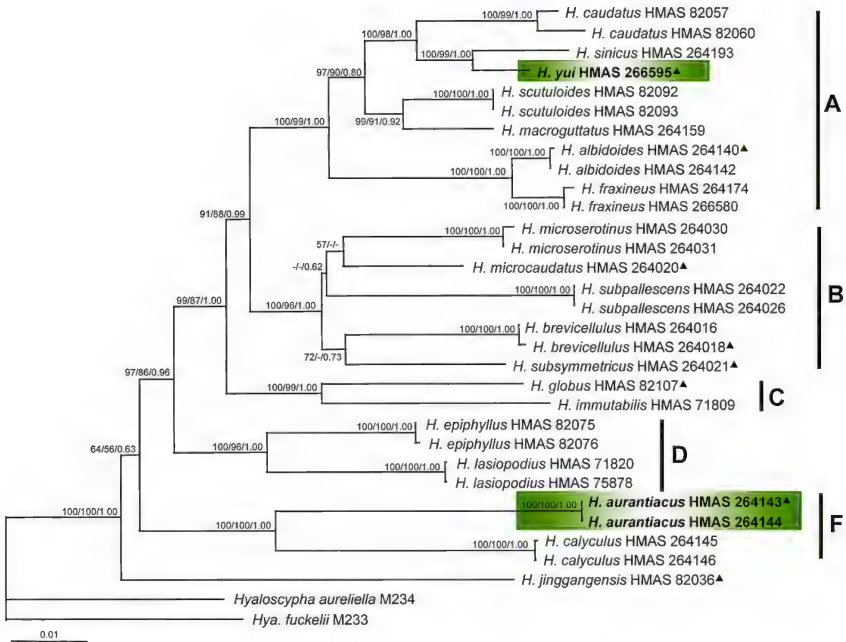

FIG. 3. Neighbor-joining tree of selected *Hymenoscyphus* species inferred from combined ITS, LSU and BTU sequences. Bootstrap values of NJ (left) and MP (middle) > 50%, and Bayesian posterior probabilities values (right) >0.50 are indicated at the nodes. ▲ indicates an ex-holotype sequence.

Taxonomy

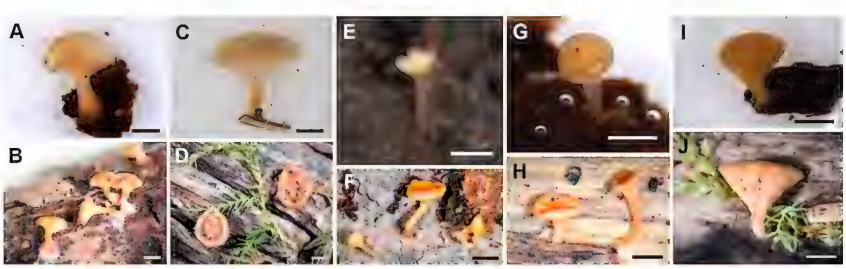

FIG. 4. Gross morphology of apothecia (A, C, E, G, I: Rehydrated apothecium; B, D, F, H, J: Dried apothecia on natural substrates). A, B. *Hymenoscyphus aurantiacus* (HMAS 264143, holotype); C, D. *H. macrodiscus* (HMAS 264158, holotype); E, F. *H. qinghaiensis* (HMAS 264175, holotype); G, H. *H. tetrasporus* (HAS 264195, holotype); I, J: *H. yui* (HMAS 266595, holotype). Scale bars: A, B, E, F, G, H = 0.5 mm; C, D, I, J = 1 mm.

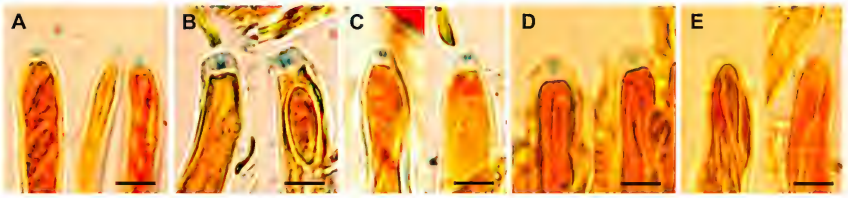

Fig. 5. IKI reaction of ascus apical ring (mounted in Lugol's solution without KOH pretreatment). A. *Hymenoscyphus aurantiacus* (holotype); B. *H. macrodiscus* (holotype); C. *H. qinghaiensis* (holotype); D. *H. tetrasporus* (holotype); E. *H. yui* (holotype). Scale bars = 5 µm.

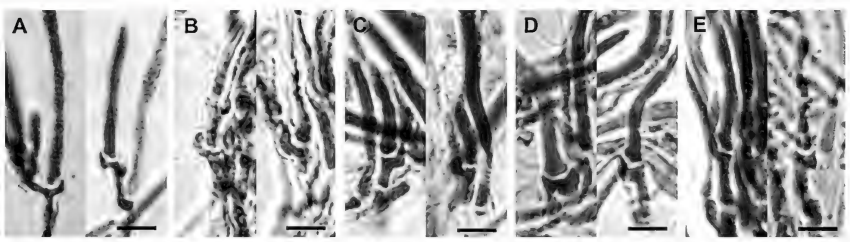

Fig. 6. Ascus bases showing croziers. A. *Hymenoscyphus aurantiacus* (holotype); B. *H. macrodiscus* (holotype); C. *H. qinghaiensis* (holotype); D. *H. tetrasporus* (holotype); E: *H. yui* (holotype). Scale bars = 5 µm.

Hymenoscyphus aurantiacus H.D. Zheng & W.Y. Zhuang, **sp. nov.** Figs 4A,B, 5A, 6A, 7

Fungal Name FN570094

Differs from *Hymenoscyphus imberbis* by its orange, distinctly stipitate apothecia and narrower excipular hyphae.

Type: China. Hubei, Wufeng, Houhe, alt. 800 m, on rotten twig and mossy wood of an unidentified deciduous tree, 12 Nov. 2004, W.Y. Zhuang & C.Y. Liu 5501 (**Holotype,** HMAS 264143).

Gene sequences ex-holotype: KJ472289 (ITS), KJ472228 (LSU), KJ472259 (BTU).

Etymology: The specific epithet refers to the color of the apothecia.

Apothecia scattered or 2–3 in a cluster, discoid, stipitate, 0.8–3 mm in diam.; hymenium surface orange, drying pale brownish to pale orange; receptacle surface paler, drying cream to cream-orange; stipe concolorous with receptacle, glabrous or nearly so, with white to orange-red mycelium at base, 0.5–1.5 mm long. Outer covering layer absent or with loosely interwoven hyphae at stipe surface, hyphae cylindrical, ends obtuse. Ectal excipulum of textura porrecta, 20–50 µm thick, cells hyaline, 10–35 × 2–6 µm. Medullary excipulum of two layers, outer layer of textura porrecta, 20–150 µm thick; inner layer of textura intricata, 30–180 µm thick; hyphae hyaline, 2–4 µm wide. Subhymenium not distinguishable. Hymenium 85–95 µm thick. Asci arising from croziers, clavate, with apex broadly subconical to rounded, 8-spored, J+, apical ring

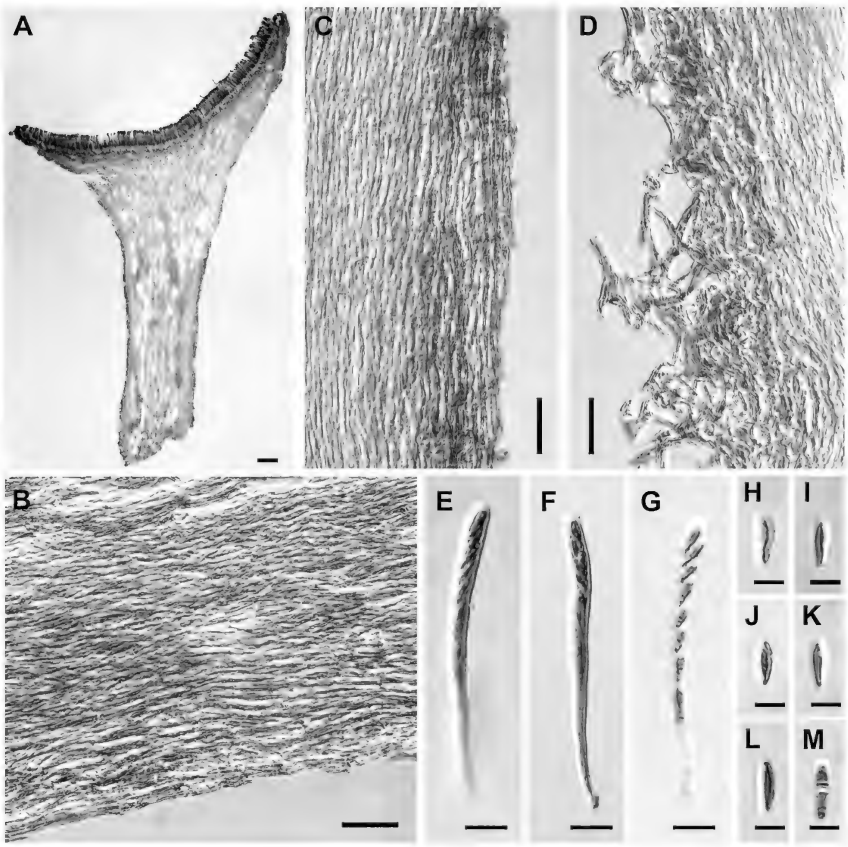

Fig. 7. *Hymenoscyphus aurantiacus* (A–C, E, F, H–K: HMAS 264143, holotype; D, G, L, M: HMAS 264144). A. Longitudinal section of apothecium; B. Excipular structure at flank; C, D. Structure at the middle of stipe; E–G. Ascus with ascospores; H–L. Ascospore; M. 1-septate ascospore. Scale bars: A = 100 μm; B–D = 20 μm; E–G = 10 μm; H–M = 5 μm.

as two blue lines without KOH pretreatment, *Hymenoscyphus*-type, 65–80 × 4.5–5.5 μm. Ascospores clavate to slipper-shaped, upper part slightly enlarged, 0(–1)-septate, hyaline, eguttulate to occasionally with tiny guttules, uniseriate to biseriate above and uniseriate below, 7.8–11 × 2.8–3.3 μm. Paraphyses filiform, hyaline, 1–2 μm wide, equal to or slightly exceeding the asci.

Additional specimen examined — **CHINA. Hubei,** Wufeng, Houhe, alt. 800 m, on rotten seeds of an unidentified plant, 12 Nov. 2004, W.Y. Zhuang & C.Y. Liu 5492 (HMAS 264144).

Notes—In the phylogenetic trees (Figs 1–3), *H. aurantiacus* clustered with *H. calyculus* (Fr.) W. Phillips (Dennis 1956) in the same clade. *Hymenoscyphus*

calyculus differs in its yellow apothecia, broader ectal excipular cells (≤10–25 × 4–12 µm), and cylindrical, biguttulate and much larger ascospores (15–22 × 3–4.5 µm) irregularly biseriate in much larger asci (120–125 × 9–10 µm; Dennis 1956).

Among other *Hymenoscyphus* species, *H. imberbis* (Bull.) Dennis resembles *H. aurantiacus* in asci and ascospores but differs in having pure white apothecia, a short and stout stipe, and broader ectal excipular cells (10–20 × 5–10 µm; Dennis 1956). The new species also resembles *H. leucopsis* (Berk. & M.A. Curtis) Dennis in stipitate apothecia and ectal excipulum structure, but *H. leucopsis* has larger apothecia (4–7 mm diam.), a pallid to copper-colored hymenium surface, and smaller asci (38–52 × 4–5 µm) and ascospores (4–9 × 1.5–2.5; Dumont 1981).

The two *H. aurantiacus* specimens share similar macroscopic and microscopic characteristics as well as identical sequence data. A minor difference observed is that the stipe surface is glabrous in the holotype (Fig. 7c) but hairy in the other collection (Fig. 7d). We treat this as infraspecific variation.

Hymenoscyphus macrodiscus H.D. Zheng & W.Y. Zhuang, **sp. nov.** Figs 4c,d, 5b, 6b, 8

Fungal Name FN570095

Differs from *Hymenoscyphus sinicus* by its parallel outer covering layer, smaller asci, and equilaterally elliptical-fusoid smaller ascospores uniseriate in the asci.

Type: China. Xinjiang, Guozigou, alt. 1400 m, on rotten wood of an unidentified deciduous tree, 11 Aug. 2003, W.Y. Zhuang & Y. Nong 4860 (**Holotype,** HMAS 264158).

Gene sequence ex-holotype: KJ472296 (ITS).

Etymology: The specific epithet refers to the large-sized apothecia.

Apothecia scattered, discoid, stipitate, 3–8 mm in diam.; hymenium surface yellow, drying ochraceous; receptacle surface paler, drying pale ochraceous; stipe slightly rough, not darkening at base, 1–8 mm long. Outer covering layer of textura intricata, up to 35 µm thick, hyphae 2–3 µm wide. Ectal excipulum of textura prismatica, tissues somewhat gelatinized, 8–30 µm thick, cells hyaline, 5–8 µm wide. Medullary excipulum of two layers, outer layer of textura porrecta, 50–110 µm thick; inner layer of textura intricata, 50–200 µm thick; hyphae hyaline, 2–3 µm wide. Subhymenium not distinguishable. Hymenium about 150 µm thick. Asci arising from croziers, subcylindrical, tapered towards base, apex subconical to subtruncate, 8-spored, J+, apical ring as two blue lines without KOH pretreatment, *Hymenoscyphus*-type, 112–150 × 6–7.5 µm. Ascospores elliptical-fusoid, pointed at both ends, hyaline, eguttulate or rarely with very tiny oil drops, obliquely uniseriate, 11–14.5 × 3.3–4.4 µm. Paraphyses filiform, hyaline, 2–3 µm wide, not exceeding the asci.

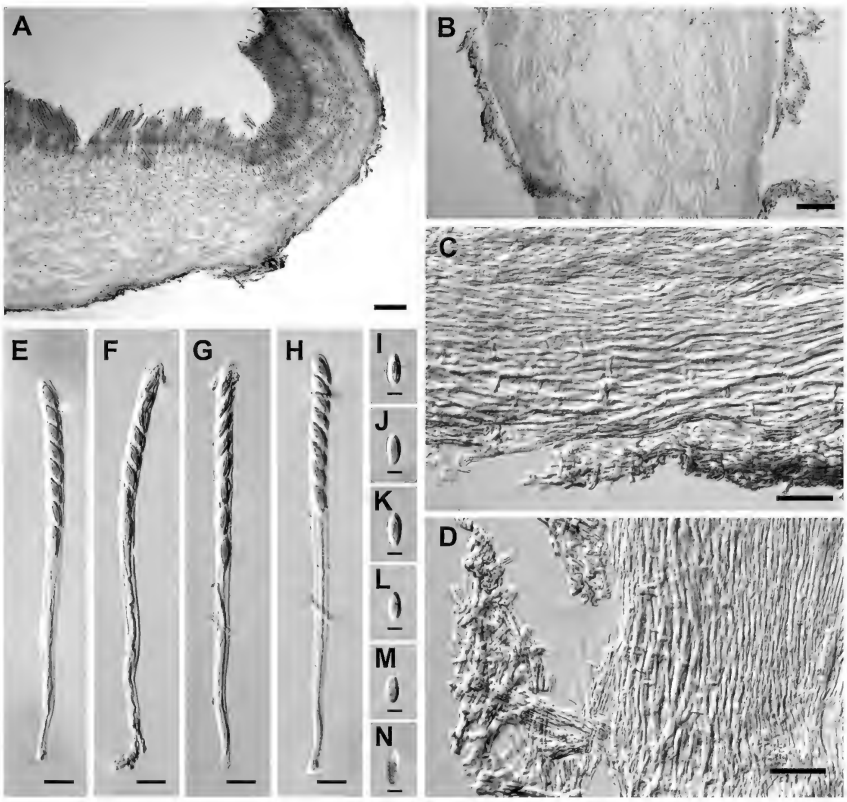

FIG. 8. *Hymenoscyphus macrodiscus* (HMAS 264158, holotype). A. Longitudinal section of portion of apothecium; B. Structure at the middle of stipe; C. Excipular structure at flank; D. Excipular structure at the middle of stipe; E–H. Ascus with ascospores; I–N. Ascospore. Scale bars: A, B = 100 μm; C, D = 20 μm; E–H = 10 μm; I–N = 5 μm.

NOTES—In the ITS phylogenetic tree (FIG. 1), *H. macrodiscus* grouped together with *H. sinicus* W.Y. Zhuang & Yan H. Zhang, which is similar in apothecial gross morphology, excipular structure, and habitat but differs in its outer covering layer composed of parallel hyphae, larger asci (125–175 × 8–12.5 μm), and larger ascospores (16–26 × 4.1–6 μm) that are inequilaterally fusoid, guttulate, and occasionally with one or two septa (Zhang & Zhuang 2002b). *Hymenoscyphus immutabilis* (Fuckel) Dennis has similar ascospores, but differs in having white and smaller apothecia (0.8–1.5 mm) with a short stipe, angular cells in ectal excipulum, absence of an outer covering layer, shorter and broader asci (80–105 × 8–10 μm), and foliicolous habitat (White 1943).

Hymenoscyphus qinghaiensis H.D. Zheng & W.Y. Zhuang, **sp. nov.** FIGS 4E,F, 5C, 6C, 9

FUNGAL NAME FN570096

Differs from *Hymenoscyphus phyllogenus* by its larger apothecia, broader ectal excipular hyphae, narrower asci arising from croziers, and narrower ascospores.

TYPE: China. Qinghai, Minhe Xigou, alt. 2600 m, on rotten leaves of *Populus* sp., 10 Aug. 2004, W.Y. Zhuang & C.Y. Liu 5248 (**Holotype**, HMAS 264175).

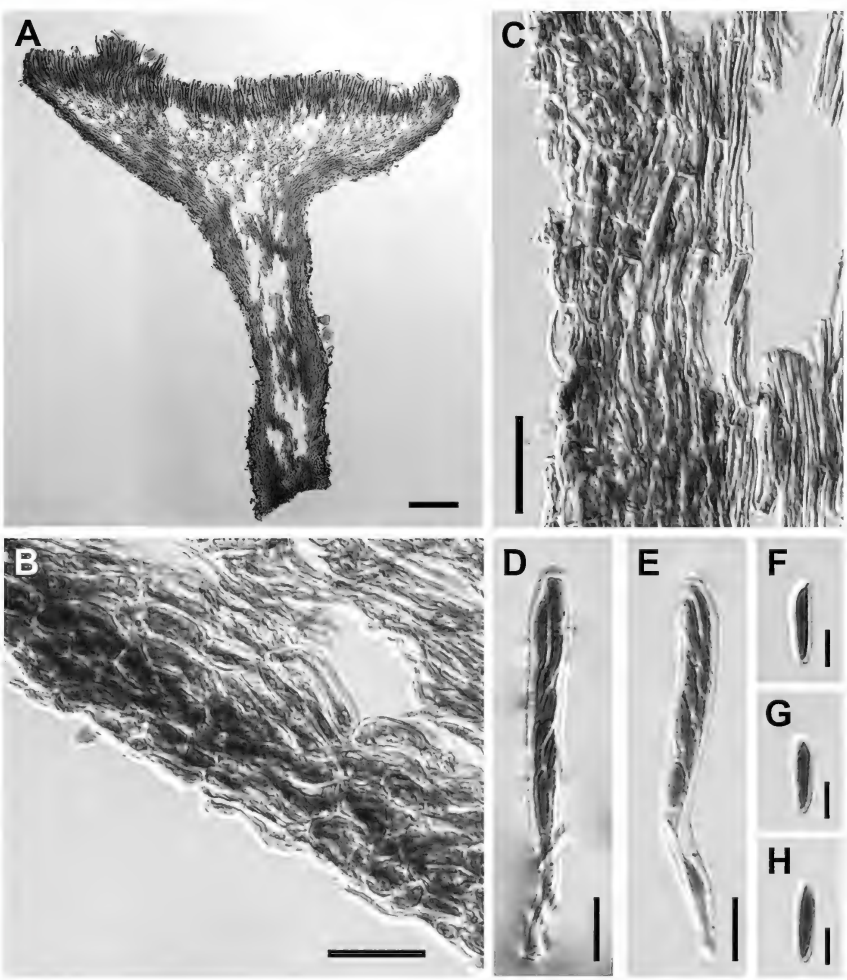

FIG. 9. *Hymenoscyphus qinghaiensis* (HMAS 264175, holotype). A. Longitudinal section of apothecium; B. Excipular structure at flank; C. Excipular structure at the middle of stipe; D, E. Ascus with ascospores; F–H. Ascospore. Scale bars: A = 100 µm; B, C = 20 µm; D, E = 10 µm; F–H = 5 µm.

GENE SEQUENCE EX-HOLOTYPE: KJ472297 (ITS).

ETYMOLOGY: The specific epithet refers to the type locality of the fungus.

Apothecia scattered, discoid to flat, stipitate, 0.4–1.4 mm in diam.; hymenium surface cream to very pale yellow, drying pale orange; receptacle surface drying cream; stipe concolorous with receptacle surface, glabrous or nearly so, not dark at base, 0.2–0.7 mm long. Outer covering layer of 1–3 hyphal layers, hyphae about 3 µm wide. Ectal excipulum of textura prismatica, 15–60 µm thick, cells hyaline, 8–30 × 5–10 µm. Medullary excipulum of two layers, outer layer of textura porrecta, 10–55 µm thick; inner layer of textura intricata, 30–140 µm thick; hyphae hyaline, 2–4 µm wide. Subhymenium not distinguishable. Hymenium 80–85 µm thick. Asci arising from croziers, clavate, apex obtuse to conical, 8-spored, J+, apical ring as two blue lines without KOH pretreatment, *Hymenoscyphus*-type, 68–89 × 6.7–7.8 µm. Ascospores cylindric-ellipsoid-fusoid, posterior end pointed, anterior end partly asymmetrical, flattened on one side, hyaline, eguttulate or rarely with very small guttules, uniseriate to biseriate above and uniseriate below, 11–14.5 × 3.3–4 µm. Paraphyses filiform, hyaline, 1.5–2.5 µm wide, not exceeding the asci.

ADDITIONAL SPECIMENS EXAMINED — **CHINA. QINGHAI,** Minhe Xigou, alt. 2600 m, on rotten leaves of *Populus* sp., 10 Aug. 2004, W.Y. Zhuang & C.Y. Liu 5241, 5247 (HMAS 264176, 264177).

NOTES—Among the known *Hymenoscyphus* species growing on rotten *Populus* leaves having similar asci and ascospores, *H. phyllogenus* (Rehm) Kuntze is most similar to *H. qinghaiensis* but differs in having much smaller apothecia (0.3–0.5 mm diam.), narrower ectal excipular cells (15–20 × 4–5 µm wide), broader asci (65–75 × 8.5–11 µm) arising from simple septa, and broader ascospores (11–14 × 3.8–5 µm; White 1943; Dennis 1956). The new species is also similar to *H. albopunctus* (Peck) Kuntze, which differs obviously in broader asci (60–70 × 8–10 µm) arising from simple septa, ascospores with 2–4 conspicuous oil drops, and growing on leaves of *Fagus* sp. (White 1943).

In the ITS phylogenetic tree (FIG. 1), *H. qinghaiensis* stands in a distinct lineage within Clade A and of uncertain relationship with other species.

Hymenoscyphus tetrasporus H.D. Zheng & W.Y. Zhuang, **sp. nov.** FIGS 4G,H, 5D, 6D, 10

FUNGAL NAME FN570098

Differs from all the known *Hymenoscyphus* species by its asci containing 4 ascospores at maturity.

TYPE: China. Hebei, Wulingshan Lianhuachi, alt. 1800 m, on herbaceous stem of an unidentified monocot, 26 Aug. 1989, W.Y. Zhuang 490 (**Holotype,** HMAS 266592).

GENE SEQUENCE EX-HOLOTYPE: KJ472302 (ITS).

ETYMOLOGY: The specific epithet refers to the number of ascospores in mature asci.

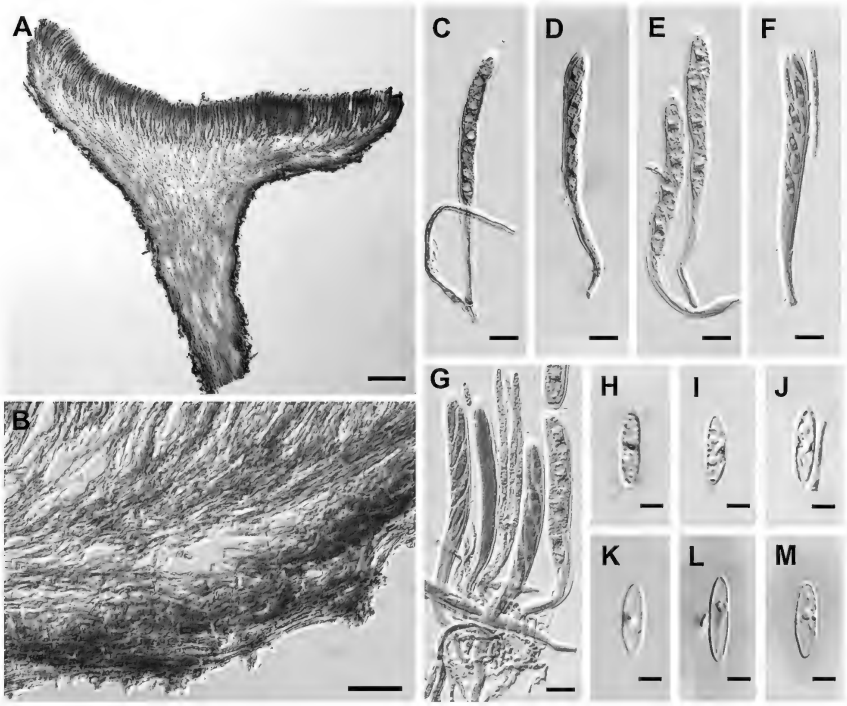

Fig. 10. *Hymenoscyphus tetrasporus* (HMAS 264195, holotype). A. Longitudinal section of apothecium; B: Excipular structure at flank; C–E: Mature ascus / asci with four ascospores; F: Immature ascus with four normal ascospores and four degenerated ones; G: Asci at different developmental stages; H–M: Ascospore. Scale bars: A = 100 μm; B = 20 μm; C–G = 10 μm; H–M = 5 μm.

Apothecia scattered, discoid, stipitate, 1–2 mm in diam.; hymenium surface yellow, drying pale brownish yellow; receptacle surface drying pallid to light orange; stipe concolorous with receptacle surface, glabrous or nearly so, 0.5–1.2 mm long. Outer covering layer about 15 μm thick, hyphae with walls roughened, about 2 μm wide. Ectal excipulum of textura prismatica, glabrous or with short hyphal protrusions, 20–50 μm thick, cells hyaline, 6–15 × 4–8 μm. Medullary excipulum of two layers, outer layer of textura porrecta, 30–40 μm thick, inner layer of textura intricata, 50–90 μm thick; hyphae hyaline, 2–3 μm wide. Subhymenium not distinguishable. Hymenium about 125 μm thick. Asci arising from croziers, subcylindrical to clavate, apex obtuse, 8-spored when young, with 4 mature spores at maturity, J+, apical ring as two blue lines without KOH pretreatment, *Hymenoscyphus*-type, 100–115 × 7–9 μm. Ascospores elliptical-fusoid with blunt ends, hyaline, having a conspicuous central dark stained area in cotton blue, with 2–4 large oil drops and several

small ones, uniseriate, (16–)17.8–22 × 4.5–5.5 µm. Paraphyses filiform, 2.5–4 µm wide, equal to or slightly exceeding the asci.

NOTES—The most distinctive character of *H. tetrasporus* is that four of the eight ascospores present in young asci abort during development. Therefore, each mature ascus contains only four ascospores (FIG. 10C–G). This prominent character easily distinguishes it from its morphologically most similar species, *H. caudatus* (P. Karst.) Dennis and *H. hyaloexcipulus* H.D. Zheng & W.Y. Zhuang.

Phylogenetically (FIG. 1) this new species grouped together with *H. macroguttatus* Baral et al. and *H. scutuloides* Hengstm., which are similar in anatomic structure, the asci arising from croziers, and guttulate ascospores. *Hymenoscyphus macroguttatus* differs in 8-spored, shorter and wider asci (77–97 × 9.5–11 µm) and shorter (13.5–18 × 4–5.5 µm) and scutuloid ascospores (Zheng & Zhuang 2013b). *Hymenoscyphus scutuloides* is distinguished by 8-spored asci and scutuloid ascospores with cilia at one or both ends (Hengstmengel 1996).

Hymenoscyphus yui H.D. Zheng & W.Y. Zhuang, **sp. nov.** FIGS 4I,J, 5E, 6E, 11

FUNGAL NAME FN570099

Differs from *H. macrodiscus* by its outer covering layer of parallel hyphae, ectal excipular hyphae slightly undulate, shorter asci, and inequilaterally elongate-ellipsoid ascospores.

TYPE: China, Xinjiang, Guozigou, alt. 1800 m, on rotten twig of unidentified deciduous tree, 11 Aug. 2003, W.Y. Zhuang & Y. Nong 4877 (**Holotype**, HMAS 266595).

GENE SEQUENCES EX-HOLOTYPE: KJ472303 (ITS), KJ472258 (LSU), KJ472287 (BTU).

ETYMOLOGY: The specific epithet honors the late distinguished Chinese mycologist, Prof. Y.N. Yu.

Apothecia scattered, discoid, stipitate, 2–4 mm in diam.; hymenium surface yellow, drying dull orange to brownish orange; receptacle surface paler, drying cream-orange; stipe concolorous with receptacle surface, glabrous or nearly so, having whitish mycelium at base, 0.5–8 mm long. Outer covering layer of 2 or more hyphal layers, hyphae 2–3 µm wide. Ectal excipulum of textura prismatica, 40–70 µm thick, hyphae slightly undulated and somewhat gelatinized, cells hyaline, 10–25 × 3–7 µm. Medullary excipulum of two layers, outer layer of textura porrecta, 20–80 µm thick; inner layer of textura intricata, 40–150 µm thick; hyphae hyaline, 2–3 µm wide. Subhymenium not distinguishable. Hymenium 110–125 µm thick. Asci arising from croziers, subcylindrical, apex rounded, 8-spored, J+, apical ring as two blue lines without KOH pretreatment, *Hymenoscyphus*-type, 86–115 × 5.5–8.5 µm. Ascospores elongate ellipsoid, slightly flattened on one side, hyaline, eguttulate to occasionally with a few tiny oil drops, uniseriate, 11–15.5 × 3.3–5 µm. Paraphyses filiform, hyaline, 1.5–2.5 µm wide, not exceeding the asci.

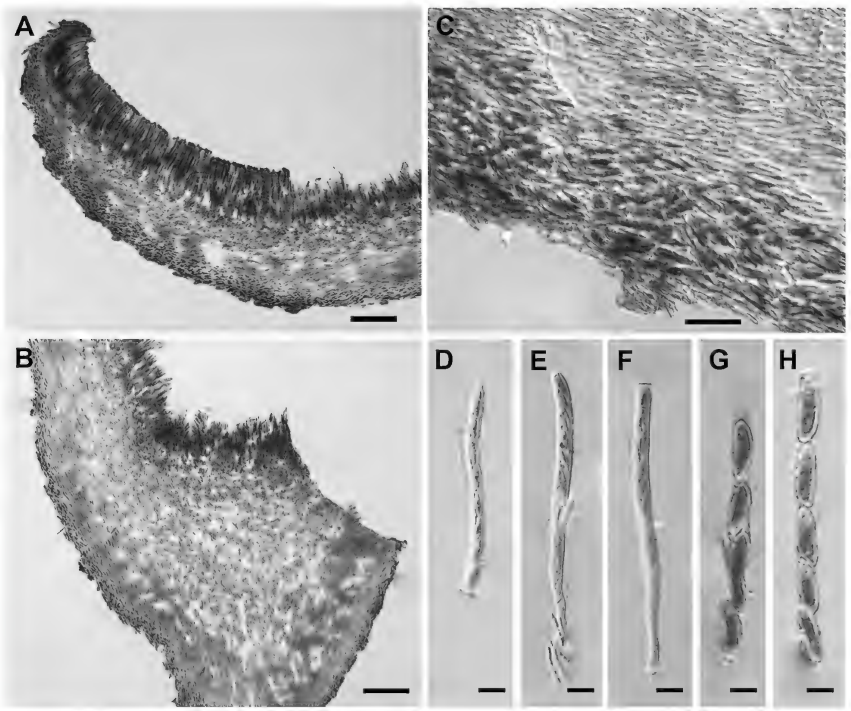

Fig. 11. *Hymenoscyphus yui* (HMAS 266594). A, B. Longitudinal section of portion of apothecium; C. Excipular structure at flank; D–F. Immature ascus; G, H. Ascospores in ascus. Scale bars: A, B = 100 µm; C = 20 µm; D–F = 10 µm; G, H = 5 µm.

Additional specimen examined — **CHINA. Xinjiang,** Yining, Qabqal, alt. 2000 m, on rotten wood of an unidentified deciduous tree, 13 Aug. 2003, W.Y. Zhuang & Y. Nong 4911 (HMAS 266594).

Notes — In the ITS tree (Fig. 1), *H. yui* appears to be a sister of *H. serotinus* (Pers.) W. Phillips, which is quite different in ectal excipulum structure, and bigger (18–28 × 3–4 µm), guttulate, strongly heteropolar and curved ascospores biseriate in asci (Dennis 1956; Baral & Bemmann 2013). In the trees inferred from LSU, BTU, and multilocus data (Figs 2,3), the new species clusters with *H. sinicus* in a highly supported subclade. *Hymenoscyphus sinicus* is similar in apothecial size and color, excipulum structure, and lignicolous habitat but differs in larger asci (125–175 × 8–12.5 µm) and guttulate ascospores, which are larger (16–26 × 4.1–6 µm; Zhang & Zhuang 2002b).

The apothecial gross morphology and habitat of *H. yui* are also similar to *H. macrodiscus*, which differs by its interwoven hyphae in the outer covering layer, straight instead of undulate hyphae in ectal excipulum, longer asci (112–150 × 6–7.5 µm), and equilaterally ellipsoid ascospores.

Discussion

In this paper, five new species of *Hymenoscyphus* are described based on morphological characters that are congruent with phylogenetic inference. Our study revealed that combining morphological with molecular data is important for defining a species. In the case of *H. aurantiacus*, the stipe surface morphology differs somewhat between the two collections as mentioned previously, yet the sequence identity and major morphological similarities support them as conspecific. The minor stipe surface differences among collections may be considered infraspecific variations.

The previous phylogenetic studies of *Hymenoscyphus* were preliminarily ITS-based using small sample sizes (Zhang & Zhuang 2004, Baral et al. 2006, Han & Shin 2008, Johnston & Park 2013, Zheng & Zhuang 2013a). Our analyses have been based on more extensive sampling and multi-gene sequence data, which provide a more comprehensive understanding of the genus. Our phylogenetic reconstruction produced six main clades within *Hymenoscyphus*. *Hymenoscyphus fructigenus*, the type species of the genus, is located in Clade A together with *H. albidoides*, *H. albidus*, *H. caudatus*, *H. dehlii*, *H. fucatus*, *H. macroguttatus*, *H. fraxineus*, *H. scutuloides*, *H. serotinus*, and *H. sinicus* in addition to four new taxa described here (*H. macrodiscus*, *H. qinghaiensis*, *H. tetrasporus, H. yui*). These fungi represent the core group of *Hymenoscyphus*. Generally, species in this clade have relatively large apothecia, rectangular cells in ectal excipulum, and scutuloid ascospores.

The seven species—*H. brevicellulus, H. ginkgonis*, *H. hyaloexcipulus, H. microcaudatus, H. microserotinus, H. subpallescens, H. subsymmetricus*—that comprise Clade B possess rectangular ectal excipular cells and scutuloid or ellipsoid ascospores usually less than 20 µm long. Clade C contains *H. crataegi*, *H. globus,* and *H. immutabilis,* which share the presence of angular, isodiametric to subglobose cells in the ectal excipulum and fusoid, symmetrical ascospores less than 15 µm long. The three taxa in Clade D share yellowish apothecia and the presence of croziers at ascus base.

Hymenoscyphus berggrenii, *H. haasticus, H. kiko*, *H. ohakune,* and *H. waikaia*, species so far known only from New Zealand (Johnston & Park 2013), comprise Clade E. They are similar in that the paraphyses branch on their upper portions to form an epithecium-like layer across the top of the asci and by occurring on *Nothofagus* leaves. The phylogenetic position of these fungi may be influenced by geographic separation and host restriction.

Hymenoscyphus aurantiacus and *H. calyculus,* which form Clade F, resemble each other in their yellow to orange apothecia and J+ asci with croziers at base. They appear to be similar to taxa in Clade D but are differentiated by their sequence data.

Analyses of individual gene sequences showed that ITS and BTU are efficient in distinguishing the species investigated, while LSU is problematic in some cases due to its limited interspecific nucleotide divergence. Based on the information gathered, it is still difficult to fully understand the phylogeny of *Hymenoscyphus*. Evolutionary relationships among species and clades need further exploration by integrated studies. Along with increasing the number of taxa and features investigated within the genus, a better and more reasonable phylogeny of *Hymenoscyphus* will be displayed.

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Phyllachora jianfengensis sp. nov. from China

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Abstract—A new species, *Phyllachora jianfengensis*, causing tar spots on leaves of *Miscanthus sacchariflorus* is reported from Hainan Province. *Phyllachora jianfengensis* differs from similar species by its longer ascospores.

Key words—*Phyllachoraceae*, *Phyllachorales*, taxonomy

Introduction

The conserved generic name *Phyllachora* Nitschke ex Fuckel was published by Fuckel (1870) with *P. graminis* (type) and 17 other species. The genus was initially defined as fungi parasitic on leaves or stems of plants and with obvious stroma. Following modern research (Doidge 1942, Parbery 1967, Cannon 1991, Pearce & Hyde 1994), the characteristics of *Phyllachora* are being parasitic and having paraphyses, unitunicate 8- or 4-spored asci, and hyaline ascospores. According to Kirk et al. (2008), *Phyllachora* belongs to *Phyllachoraceae*, *Phyllachorales*, and includes about 944 species worldwide.

An undescribed *Phyllachora* species was reported on leaves of *Miscanthus sacchariflorus* collected from Hainan Province, China, in 2007. It differs morphologically from other *Phyllachora* species on three genera of *Andropogoneae* subtribe *Saccharinae* (*Imperata*, *Miscanthus*, and *Saccharum*) and is described here as *Phyllachora jianfengensis*.

Materials & methods

We measured the length and width of leaf spots with a ruler and observed the presence of holes with a magnifying glass. The characters of asci were observed after they were separated from ascomata with dissecting needle and stained in lactophenol blue solution. The asci and ascospore were measured using a C7 ocular micrometer and Nikon E200MV photomicroscope. We prepared slices by using a Leica CM1850UV

freezing microtome. The holotype of the new species is deposited in the Herbarium Mycologicum Academiae Sinicae, Beijing, China (HMAS).

Taxonomy

Phyllachora jianfengensis Na Liu & M.Z. Li, **sp. nov.** PLATE 1

FUNGAL NAME FN570152

Differs from *Phyllachora ischaemi, P. miscanthi, P. miscanthidii,* and *P. tengchongensis* by its longer ascospores.

TYPE: China, Hainan Province, Jianfengling, alt. 900 m, on *Miscanthus sacchariflorus* (Maxim.) Hack. (*Poaceae*), 18 XI 2007, S.H. He, L. Guo & Z. Li 2014 (HMAS 245921, **holotype**).

ETYMOLOGY: From Jianfengling mountain, where the new species was collected.

Leaf spots immersed in the mesophyll on ellipsoidal glossy black leaf spots 0.6–3 mm in diam, surrounded by pale yellow haloes, visible both sides of leaves, sparse, 1–4-loculate, subglobose, ostiolate. ASCOMATA 202–278 × 180–213 µm, immersed in the mesophyll layer of the leaves, subglobose or ellipsoidal, upper wall up to 25 µm thick composed of black material, lateral wall up to 18 µm thick composed of epidermal cells and black material, and lower wall up to 13 µm thick composed of parenchyma cells; asci rising from the basal and lateral wall of the ascoma. Paraphyses 1–2 µm wide, filiform, aseptate, unbranched, shorter than asci, thin-walled. ASCI 83–125 × 11–26 µm, cylindrical to clavate, apex round or slightly sharp, thin-walled, 8-spored, long pedunculate or without peduncle, unitunicate. ASCOSPORES 32–37 × 5–9 µm, biseriate or arranged irregularly, long ellipsoidal with slightly sharp apex to oblong, unicellular, hyaline, smooth. ANAMORPH not seen.

COMMENTS—Species of *Phyllachora* with ascospores longer than 30 µm are uncommon. Based on the assumption that *Phyllachora* species are likely to be host-specific, we compared our collection with *Phyllachora* species reported from the related hosts *Miscanthus*, *Imperata*, and *Saccharum* (Chen 2007, Hodkinson et al. 2002).

Four *Phyllachora* species have been recorded on *Miscanthus*: *P. ischaemi* Syd. & P. Syd. with ovato-ellipsoidal ascospores (12–15 × 5–6.5 µm) and cylindraceo-clavate asci (45–60 × 12–18 µm; Sydow & Sydow 1915), *P. miscanthi* Syd. & P. Syd. with broadly ellipsoidal ascospores (18–24 × 12–14 µm) and cylindriceo-clavate asci (160–180 × 18–22 µm; Sydow & Sydow 1917), *P. miscanthidii* Doidge with ellipsoidal to subclavate ascospores (22–27.5 × 7.5–10 µm) and cylindrical to cylindrico-elliptical asci (125 × 17–20 µm; Doidge 1942), and *P. tengchongensis* Na Liu & L. Guo with narrowly ellipsoid ascospores (15–26 × 5–10 µm) and clavate asci (93–159 × 15–23 µm;

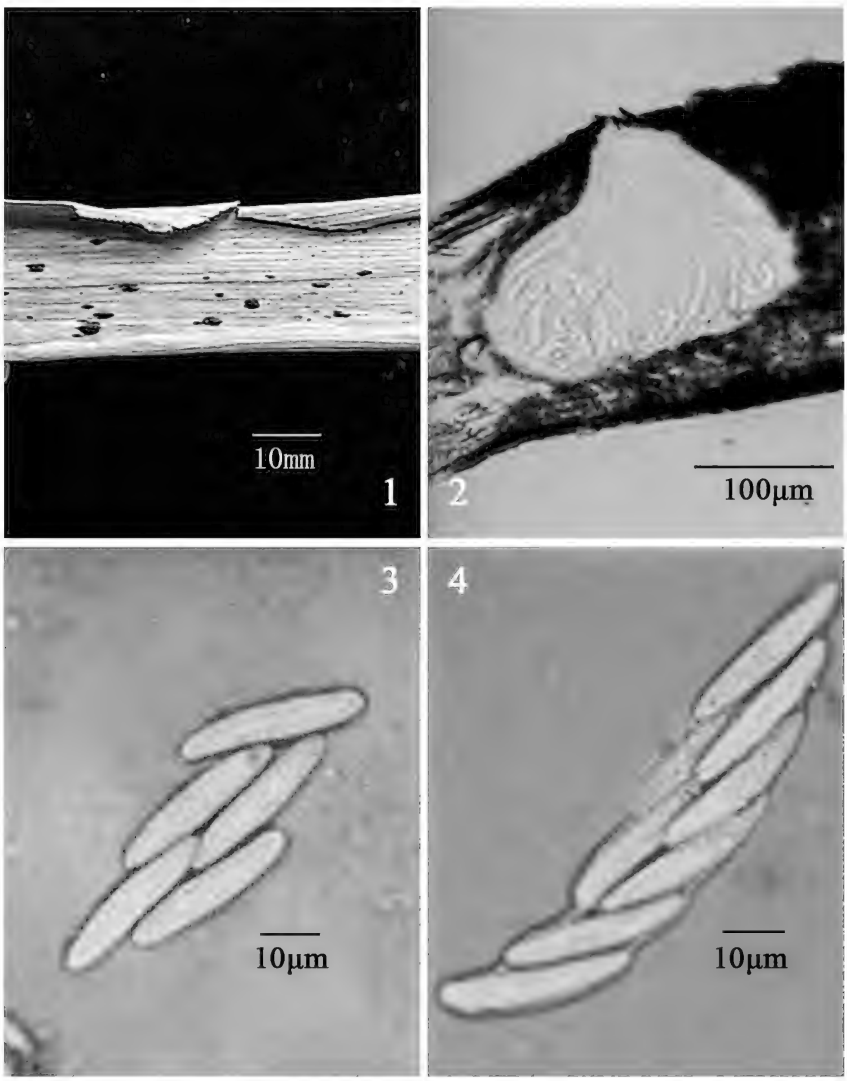

Plate 1. *Phyllachora jianfengensis* (HMAS 245921, holotype).
1. Leafspots; 2. Ascomata; 3, 4; Asci and ascospores.

Liu et al. 2007). These species all differ from *P. jianfengensis* by their much shorter ascospores; additionally, *P. ischaemi* differs by its shorter asci and *P. miscanthi* by its longer asci and wider ascospores.

Two *Phyllachora* species have been reported on *Imperata*: *P. oxyspora* Starbäck (= *P. imperatae* Syd. & P. Syd., = *P. antioquensis* Chardón, and = *P. sorghastri* Chardón; Species Fungorum 2015) with clavulate ascospores (20–25 × 5–6 µm) and cylindrical asci (96–115 × 9–10 µm; Starbäck 1899) and "*P. imperaticola*" Sawada (an invalid name, lacking a Latin description) with ellipsoidal ascospores (17–19 × 9–10 µm) and clavate-cylindrical asci (89–110 × 13–18 µm; Sawada 1959). Both species differ from *P. jianfengensis* by their much shorter ascospores as well as their host specialisation; and *P. oxyspora* additionally differs by its narrower asci.

Two *Phyllachora* species have been reported on *Saccharum*: *P. sacchari-spontanei* Syd. & P. Syd. with fusiform ascospores (22–27 × 7.5–8.5 µm) and clavate asci (75–100 × 18–22 µm; Sydow & Sydow 1913) and *P. sacchari* Henn. (= *P. sacchari-aegyptiacae* Briosi & Cavara, = *P. sorghi* Höhn., = *P. rottboelliae* Syd. et al., and = *P. andropogonicola* Speg.; Species Fungorum 2015) with ellipsoid to ovoid ascospores (12–18 × 9–12 µm) and clavate asci (80–120 × 15–18 µm; Hennings 1902). Both species differ from *P. jianfengensis* by their much shorter ascospores as well as by their host specialisation, and *P. sacchari* additionally differs by its wider ascospores.

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A new species and a new combination in *Codinaea* from Brazil

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Abstract — *Codinaea aquatica* sp. nov., a hyphomycete collected on submerged decaying branches of an unidentified plant, is described and illustrated. It is distinguished by monophialidic and polyphialidic conidiogenous cells that produce fusiform to navicular unicellular hyaline conidia with a single unbranched setula at each end, basal setula strongly curved, apical setula straight or flexuous. *Codinaea tropicalis* is proposed as a comb. nov. based on *Dictyochaeta tropicalis*.

Key words — freshwater, asexual fungi, systematics

Introduction

Codinaea introduced by Maire (1937) with *C. aristata* Maire as the type species is characterized by distinct, single, mononematous or synnematous, brown to pale brown conidiophores, and an acerose to subulate, septate, dark brown, thick-walled seta that may occur in some species. The conidiogenous cells are mono- or polyphialidic, terminal, determinate, or with a few sympodial extensions and sometimes with enteroblastic percurrent regenerations and with distinct cylindrical or funnel-shaped flaring, sometimes collapsed collarettes at the conidiogenous loci and producing unicellular or septate, falcate to lunate hyaline conidia with a filiform appendage at each end (Almeida & Gusmão 2014,

Granados et al. 2014, Hughes & Kendrick 1968, Li et al. 2012, Réblová & Winka 2000, Seifert et al. 2011). For two decades (Gamundi et al. 1977), the genus *Dictyochaeta* (Spegazzini 1923) was considered an earlier name for *Codinaea* (Maire 1937), but following the molecular analyses of Réblová & Winka (2000), both genera are currently accepted. Li et al. (2012) recommended that species with filiform appendages be retained in *Codinaea* and taxa lacking appendages be placed in *Dictyochaeta*.

During a mycological survey of freshwater fungi associated with submerged plant materials in a river at a Brazilian Atlantic forest a conspicuous fungus was collected that is described here as a new *Codinaea* species.

Materials & methods

Individual collections were placed in flasks and plastic bags, taken to the laboratory, and treated according to Castañeda (2005). Mounts were prepared in polyvinyl alcohol-glycerol (8 g PVA in 100 ml of water, plus 5 ml of glycerol) and in lactic acid (90%), and measurements were made at a magnification of ×1000 under a Nikon Eclipse Ni-U microscope with bright field optics. Photomicrographs were obtained with the Nikon microscope with a Nikon digital camera (model DS-Fi2). The holotype was deposited in the Herbarium of Universidade Federal de Pernambuco, Recife, Brazil (URM).

Taxonomy

Codinaea aquatica R.F. Castañeda, M.S. Oliveira & Malosso, **sp. nov.** FIG. 1

MycoBank MB812851

Differs from all other *Codinaea* species by its fusiform to navicular conidia with a strongly curved, shorter basal appendage and a straight or flexuous, longer apical appendage.

TYPE Brazil, Pernambuco, Cabo de Santo Agostinho, Refúgio de Vida Silvestre, Matas do Sistema Gurjaú, 8°13′S 35°03′W, alt. 85 m., on submerged decaying branches of unidentified plant in a river, 12.V.2015, coll. M.S. Oliveira (**Holotype**, URM 87707).

ETYMOLOGY: Latin, *aquatica*, referred to its growing in water.

COLONIES on the natural substrate scattered, hairy, black. Mycelium superficial and immersed, composed of septate, branched, brown, smooth-walled hyphae 2–4 µm diam. SETAE absent. CONIDIOPHORES distinct, erect, straight or flexuous, cylindrical, 4–7-septate, brown to dark brown below, pale brown to brown toward the apex, 200–350 × 4–6 µm, smooth. CONIDIOGENOUS CELLS mono-and polyphialidic, integrated, 40–60 × 6–7 µm, terminal, mostly determinate, but sometimes indeterminate with few sympodial extensions, brown to pale brown, with 1–2 funnel-shaped collarettes, 2.5–4 µm deep. CONIDIA acrogenous, fusiform to navicular, sometimes unilateral ventricose, slightly truncate at the base, obtuse at the apex, unicellular, 25–28 × 10–12 µm, thick-walled, hyaline, smooth, with a filiform appendage at each end: basal

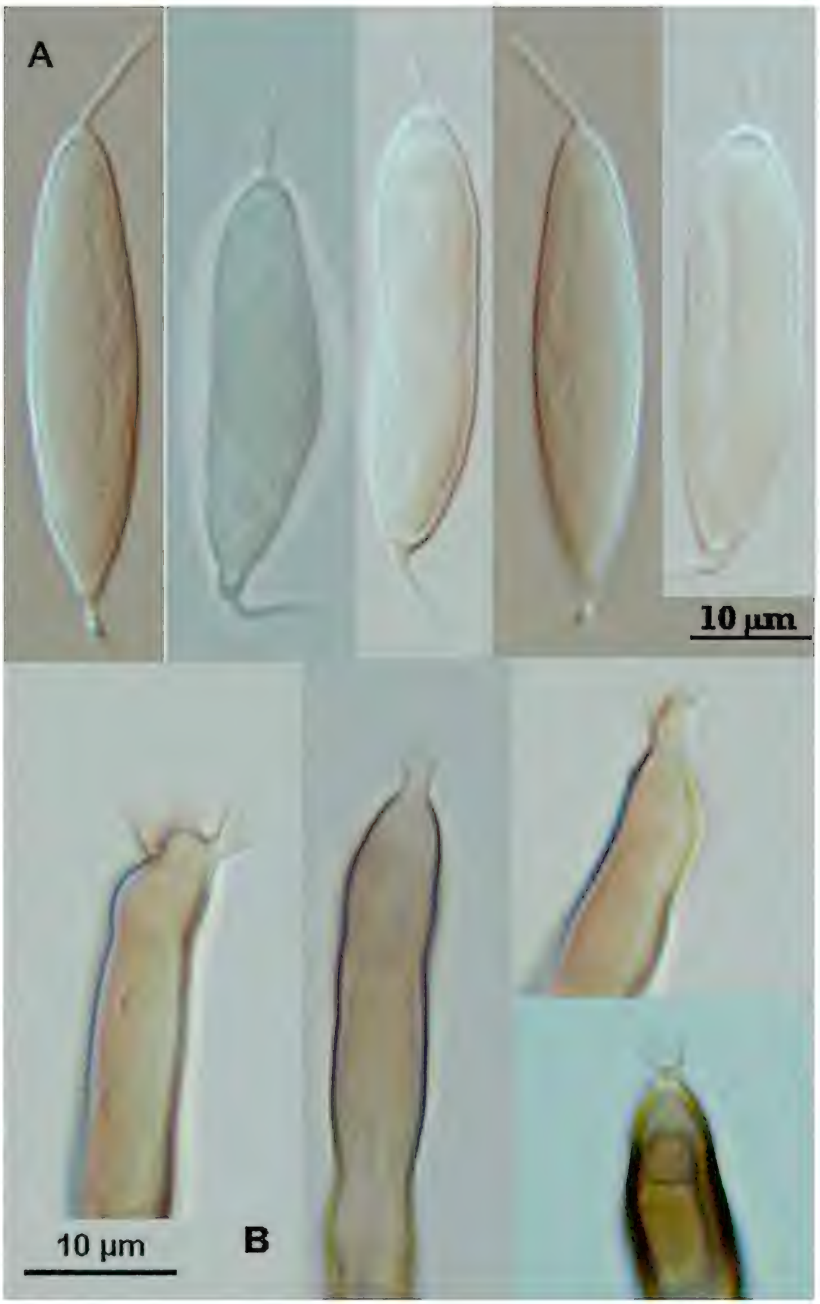

Fig. 1. *Codinaea aquatica* (ex holotype URM 87707). A. Conidia. B. Conidiogenous cells.

appendage strongly curved to forming a 90° angle with the axis of conidial body, 4.5–6 µm long, apical appendage straight or flexuous, 7–8 µm long.

Notes: Among all recorded *Codinaea* and *Dictyochaeta* species (Index Fungorum 2015) only *Dictyochaeta tropicalis* resembles *Codinaea aquatica* in the placement and arrangement of the filiform appendage. However, *D. tropicalis* [recombined in *Codinaea*, below] has ellipsoid to drop-shaped conidia (7.5–9.5 × 3–5 µm; Bhat & Kendrick 1993), which cannot be confused with those of *C. aquatica*.

Codinaea tropicalis (Bhat & W.B. Kendr.) R.F. Castañeda & Malosso, **comb. nov.**

MycoBank MB812854

≡ *Dictyochaeta tropicalis* Bhat & W. B. Kendr., Mycotaxon 49: 45 (1993).

This species has monophialidic conidiogenous cells with a flared apical collarette, and its conidia are ellipsoid to drop-shaped, hyaline, and with a filiform appendage at each end (Bhat & Kendrick 1993). The new combination is proposed because *Dictyochaeta tropicalis* has characters that fit the modern generic concept of *Codinaea* (Li et al. 2012, Réblová & Winka 2000, Seifert et al. 2011).

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Conidial fungi on *Araucaria angustifolia:* *Trichoconis foliicola* sp. nov. and two new records from Brazil

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Abstract —*Trichoconis foliicola*, a new species found on decaying needle-like leaves of *Araucaria angustifolia*, is described and illustrated. *Trichoconis antillana* and *T. queenslandica* are newly recorded from Brazil. A key to all *Trichoconis* species and illustrations of their conidia are provided.

Key words —hyphomycetes, leaf litter, taxonomy

Introduction

Trichoconis Clem., typified by *T. caudata* (Appel & Strunk) Clem., is characterized by conidiophores that are distinct, colourless, single or branched, with conidiogenous cells that are polyblastic, sympodial, denticulate, and with cylindrical denticles that are separating cells in which rhexolytic secession occurs. The conidia are solitary, fusiform, obclavate, clavate, ellipsoid, subcylindrical, or navicular, rostrate, apical and lateral, septate, and colourless (Deighton & Pirozynski 1972). The genus comprises 21 described species (Index Fungorum 2015; Clements 1909, Pavgi & Singh 1966, Deighton & Pirozynski 1972, Pirozynski 1974, Hawksworth 1980, Hoog & Oorschot 1985, Matsushima 1989, 1993, Castañeda-Ruiz & Kendrick 1991, Castañeda-Ruiz et al. 1997; Baker et al. 2001, Seifert et al. 2011, Brackel 2014). We exclude

two *Trichoconis* species from our treatment: *Trichoconis echinophila* (Hoog & Oorschot 1985) is considered to belong in *Pseudotrichoconis* (Baker et al. 2001, Seifert et al. 2011), and *Trichoconis indica* (Pavgi et al. 1966) was described and illustrated as having terminal integrated, unilocal conidiogenous cells, lacking separating cells, and having schizolytic conidial secession, characters that are unquestionably incompatible with the *Trichoconis* generic concept (Seifert et al. 2011).

During research on hyphomycetes occurring on decaying needle-like leaves of *Araucaria angustifolia* (Bertol.) Kuntze (*Araucariaceae*) we noted a unique specimen of *Trichoconis,* which we describe here as new. We also present a synopsis of accepted species and their conidia (Figs 3, 4).

Materials & methods

Samples of decaying needles of *A. angustifolia* were collected, placed in paper bags, transported to the laboratory, washed in tap water, and incubated in moist chambers at 25°C for 30 days (Castañeda-Ruiz 2005). Slide mounts were prepared in PVL (polyvinyl alcohol, lactic acid, and phenol), and micrographs were obtained with an Olympus microscope BX 51 with differential interference contrast (DIC) DP25. Specimens were deposited in the Herbarium (HUEFS).

Taxonomy

Trichoconis foliicola S.S. Silva, Gusmão & R.F. Castañeda, **sp. nov.** Fig. 1

MycoBank MB 812636

Differs from all other *Trichoconis* spp. by its narrow obclavate 2–septate conidia with flagelliform and slightly curved to recurved apical cells.

Type: Brazil. Rio Grande do Sul State: São Francisco de Paula, Floresta Nacional de São Francisco de Paula, 29°25′S 50°23′W, alt. 840 m, on decaying needle-like leaves of *Araucaria angustifolia*, 14.V.2014, coll. S.S. Silva (**Holotype**: HUEFS 211338).

Etymology: Latin, *foliicola*, growing on leaves.

Colonies on the natural substrate effuse, hyaline. Mycelium mostly superficial. Hyphae occasionally branched, smooth, hyaline, 2–3 µm wide. Conidiophores distinct, single, erect, straight to slightly flexuous, smooth, 1–2-septate, 9–31 × 2.5–3 µm. Conidiogenous cells mono- or polyblastic, integrated, terminal, cylindrical, hyaline, 8–15 × 2.5 µm, with conspicuous, cylindrical denticles, 2.5–4 µm long. Conidial secession rhexolytic. Conidia narrowly obclavate, rostrate, 2-septate, smooth, dry, hyaline, 25–50 × 2.5–4 µm, rostrum flagelliform, slightly curved to recurved, 15–25 µm long.

Notes: *Trichoconis caudata*, *T. lichenicola* D. Hawksw., and *T. queenslandica* are somewhat similar to *T. foliicola* but are easily differentiated as follows: *T. caudata* conidia are fusiform, mostly 5-septate, and 27–40 × 6.5–12 µm;

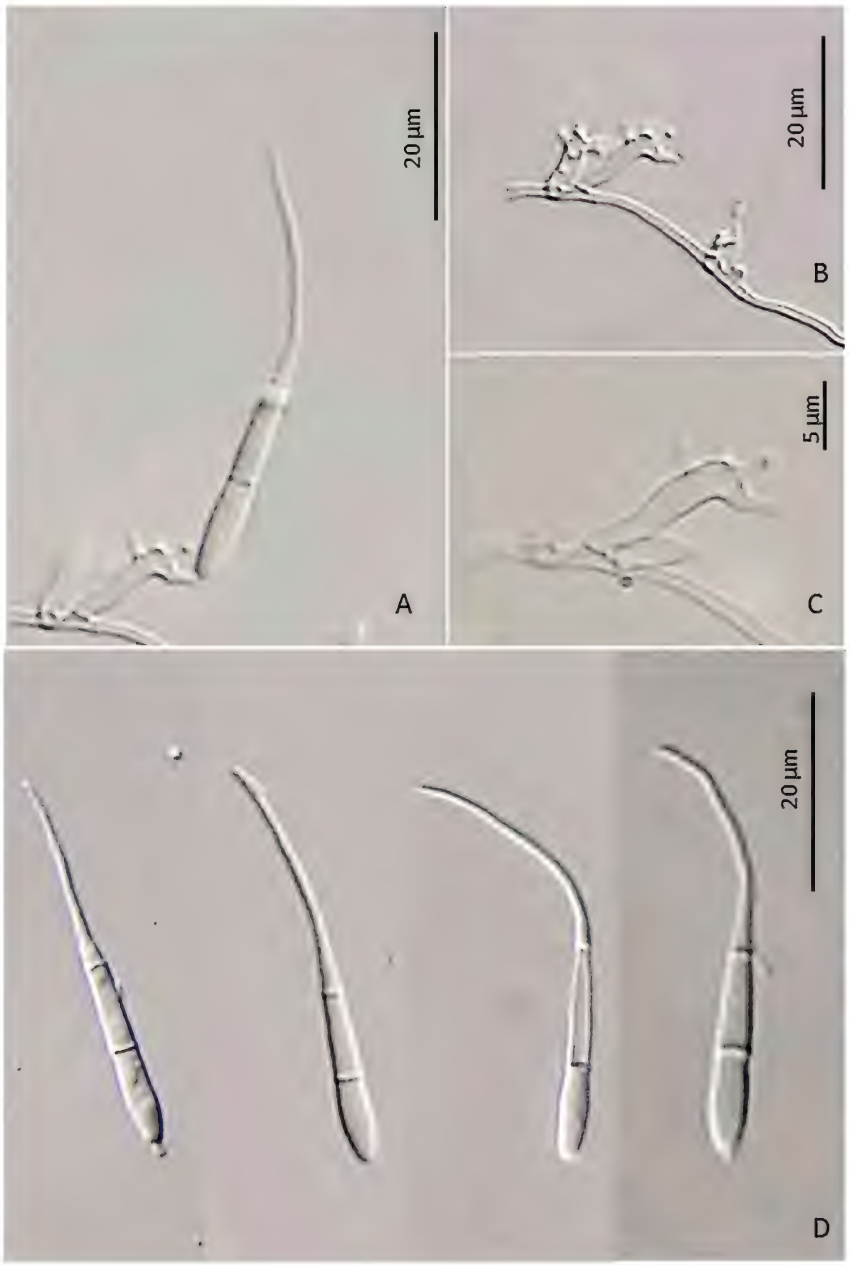


Fig. 1. *Trichoconis foliicola* (HUEFS 211338): A. Conidiophore and conidia. B. Conidiophores reduced to conidiogenous cells. C. Conidiogenous cells. D. Conidia.

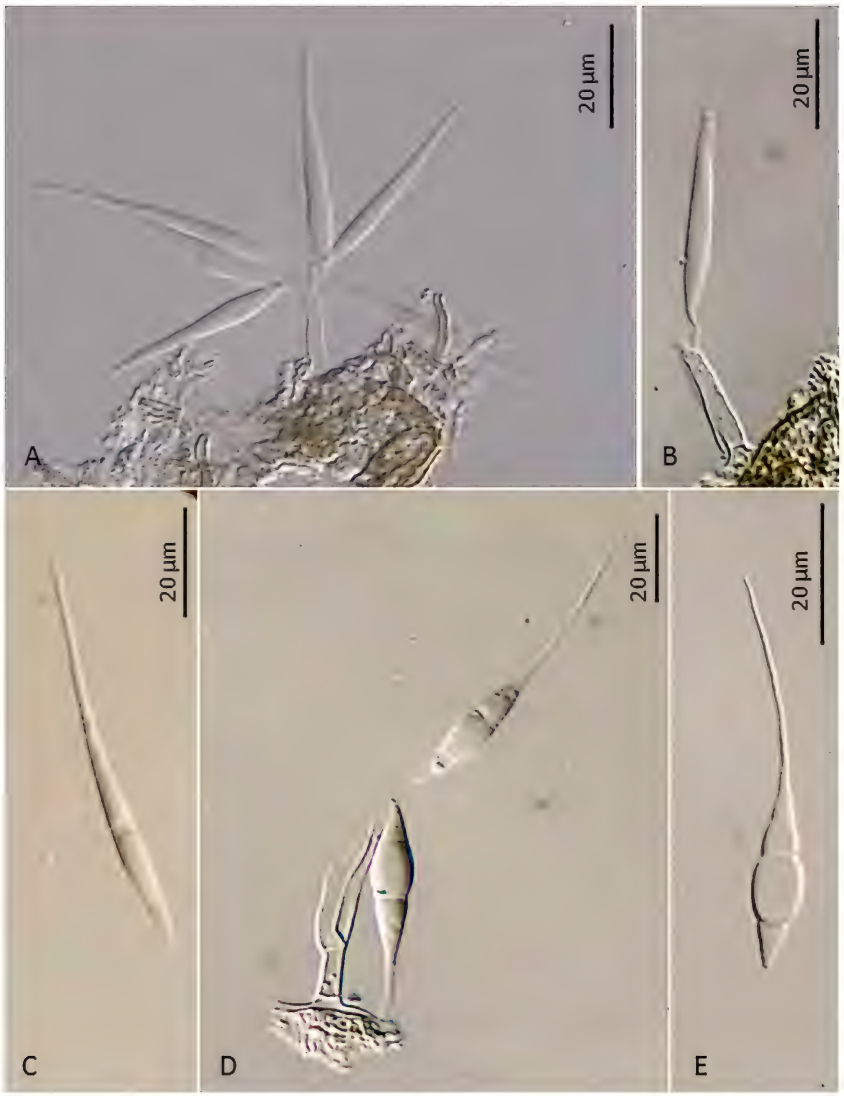


Fig 2. ***Trichoconis antillana*** (HUEFS 211339): A, B. Conidiophore and conidia. C. Conidium. ***T. queenslandica*** (HUEFS 211341): D. Conidiophore and conidia. E. Conidium.

T. lichenicola conidia are fusiform to obclavate, 2–4-septate, and 35–65 × 5.5–7 µm; and *T. queenslandica* conidia are fusiform, mostly 3-septate, and 44–85 × 5–8 µm.

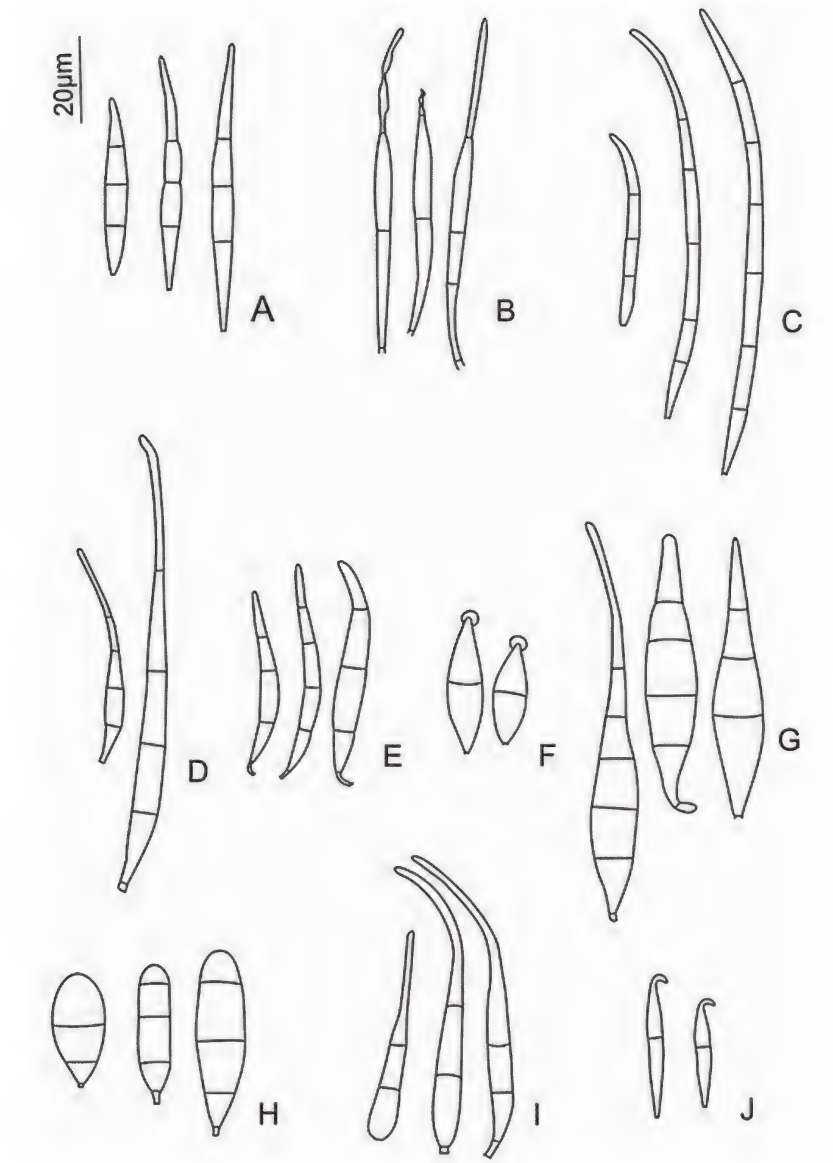
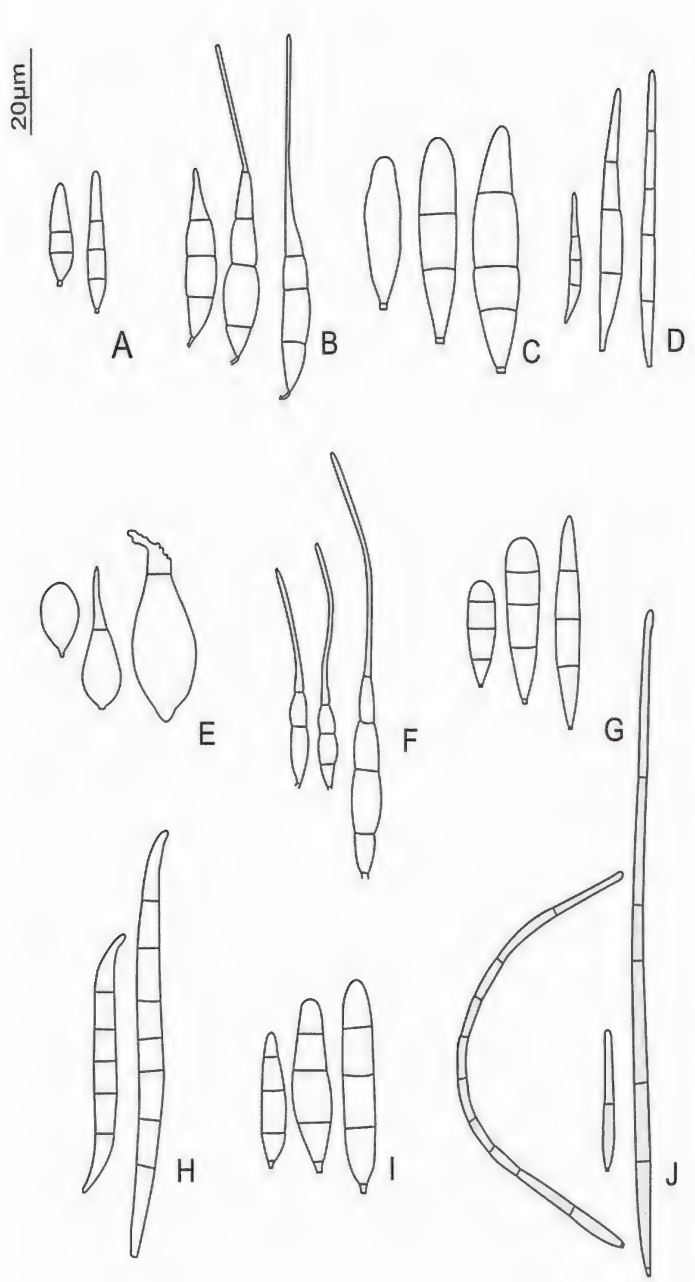


Fig 3. Conidia of *Trichoconis* spp.: A. ***T. africana*** (Deighton & Pirozynski 1972). B. ***T. amazonensis*** (Matsushima 1993). C. ***T. angustispora*** (Deighton & Pirozynski 1972). D. ***T. antillana*** (Castañeda-Ruiz et al. 1997). E. ***T. appendiculata*** (Deighton & Pirozynski 1972). F. ***T. capitata*** (Pirozynski 1974). G. ***T. caudata*** (Deighton & Pirozynski 1972). H. ***T. englerulae*** (Deighton & Pirozynski 1972). I. ***T. foliicola*** (this work). J. ***T. hamata*** (Deighton & Pirozynski 1972).


20µm
A
B
C
D
E
F
G
H
I
J

Trichoconis antillana R.F. Castañeda, W.B. Kendr. & Guarro, Mycotaxon 65: 103 (1997) Fig. 2A–C

Conidiophores 20–25 × 3–3.5 µm. Conidiogenous cells 15–20 × 3–4 µm, denticles 4–5 µm long. Conidia 45–64 × 3–4 µm.

Specimens examined: **BRAZIL, Santa Catarina State:** Urubici, Parque Nacional de São Joaquim, 28°04′S 49°25′W, 990 m alt., on decaying needle-like leaves of *Araucaria angustifolia*, 18.VIII.2014, coll. S.S. Silva (HUEFS 211339). **Rio Grande do Sul State:** São Francisco de Paula, Floresta Nacional de São Francisco de Paula, 29°25′S 50°23′W, alt. 840 m, on decaying needle-like leaves of *Araucaria angustifolia*, 14.V.2014, coll. S.S. Silva (HUEFS 211340).

Note: A new record for Brazil.

Trichoconis queenslandica Matsush., Matsush. Mycol. Mem. 6: 43 (1989) Fig. 2D–E

Conidiophores 34–67 × 3–4.5 µm. Conidiogenous cells 19–30 × 3–4.5 µm, denticles 4.5–10 µm long. Conidia 42–73 × 4.5–6 µm.

Specimen examined: **BRAZIL. Santa Catarina State:** Urubici, Parque Nacional de São Joaquim, 28°04′S 49°25′W, alt. 990 m, on decaying needle-like leaves of *Araucaria angustifolia*, 29.V.2014; coll. S.S. Silva (HUEFS 211341).

Note: A new record for Brazil.

Key to *Trichoconis* species

1. Conidiophores and conidia pale olivaceous, obclavate to filiform, 1–10-septate, 30–200 × 3–4.5 µm ..... *T. viridula*
   Conidiophores and conidia colorless 2
2. Conidia with rostrate apical cell 3
   Conidia without rostrate apical cell 9
3. Conidia with hamate rostrum, 1-septate, 20–30 × 2–3.5 µm, rostrum ≤4 µm ..... *T. hamata*
   Conidia without hamate rostrum 4
4. Conidia clavate, 1–2-septate, 36–49 × 2.5–3.5 µm, rostrum flagelliform, 17–38 µm long ..... *T. amazonensis*
   Conidia not as above 5
5. Conidia 2-septate, obclavate, 25–50 × 2.5–4 µm, rostrum flagelliform, 15–25 µm long ..... *T. foliicola*
   Conidia more than 2 septate 6

Fig 4. Conidia of *Trichoconis* spp.: A. ***T. hibernica*** (Deighton & Pirozynski 1972). B. ***T. lichenicola*** (Hawksworth 1980). C. ***T. malloti*** (Deighton & Pirozynski 1972). D. ***T. pedicephora*** (Castañeda-Ruiz & Kendrick 1991). E. ***T. physciicola*** (Brackel 2014). F. ***T. queenslandica*** (Matsushima 1989). G. *T. schiffnerulae* (Deighton & Pirozynski 1972). H. ***T. sigmoidea*** (Deighton & Pirozynski 1972). I. ***T. trichiliae*** (Deighton & Pirozynski 1972). J. ***T. viridula*** (Deighton & Pirozynski 1972).

6. Conidia 0–3-septate, mostly 3 septa, fusiform, 44–85 × 5–8 µm, rostrum 17–40 µm long ..... *T. queenslandica*
   Conidia sometimes more than 3-septate 7
7. Conidia not more than 4-septate 8
   Conidia 3–5-septate, mostly 5-septate, fusiform, 27–40 × 6.5–12 µm, rostrum 9.5–30 µm long ..... *T. caudata*
8. Conidia 2–4-septate, 35–65 × 5.5–7 µm, fusiform to obclavate, rostrum up to 30 µm long ..... *T. lichenicola*
   Conidia 4-septate, obclavate, 50–120 × 4–6 µm ..... *T. antillana*
9. Conidia with not more than one septum 10
   Conidia with more than one septum 11
10. Conidia navicular, with an apical droplet of mucilage, 1-septate, 18–26 × 6–7 µm ..... *T. capitata*
    Conidia ellipsoid to suborbicular, 0–1-septate, 10–44 × 7–11µm ..... *T. physciicola*
11. Conidia filiform, attenuated at the ends, 3–6-septate, 70–110 × 2.5–4 µm ..... *T. angustispora*
    Conidia not filiform 12
12. Conidia with not more than 3 septa 13
    Conidia with more than 3 septa 18
13. Conidial length <40 µm 14
    Conidial length >40 µm 15
14. Conidia 21–30 × 3–4 µm, 2–3-septate ..... *T. hibernica*
    Conidia 24–38 × 6.5–9.5 µm, subcylindrical, clavate-ellipsoid or obclavate-ellipsoid, 3-septate ..... *T. englerulae*
15. Conidial length <45 µm 16
    Conidial length >45 µm 17
16. Conidia 27–40 × 4–6.5 µm, obclavate to fusiform, 3-septate ..... *T. trichiliae*
    Conidia 20–41 × 5–8 µm, obovoid or cylindrical-obovoid, 3-septate ..... *T. schiffnerulae*
17. Conidia 20–50 × 5–9 µm, mostly fusiform, 0–3-septate ..... *T. malloti*
    Conidia 40–72 × 4.5–7 µm, fusiform, mostly 3-septate ..... *T. africana*
18. Conidia with not more than 4 septa 19
    Conidia 3–9-septate (mostly 5–8-septate), fusiform, sigmoid, 50–80 × 5–7 µm ..... *T. sigmoidea*
19. Conidia 3–4-septate, fusiform, 43–56 × 4–6 µm ..... *T. appendiculata*
    Conidia 2–4-septate, fusiform or obclavate, 20–52 × 3–7 µm ..... *T. pedicephora*

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Entyloma scandicis, a new smut fungus on *Scandix verna* from Mediterranean forests of Israel

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Abstract — The morphology and phylogeny of a species of *Entyloma* on *Scandix verna* (*Apiaceae, Apioideae, Scandiceae*) collected in the Mount Carmel and Lower Galilee regions of Israel were studied using light microscopy and ITS rDNA sequence analyses. The fungus differs morphologically from other species on hosts of subfamily *Apioideae: E. bupleuri, E. helosciadii* s.l., *E. kundmanniae, E. magocsyanum,* and *E. pastinacae*. Molecular phylogenetic analyses revealed that all specimens of *Entyloma* from *Scandix* represent a monophyletic lineage, sister to *E. magocsyanum*. As a result, the smut in leaves of *Scandix verna* is described and illustrated here as a new species, *Entyloma scandicis*.

Key words — *Entylomatales*, evolution, plant pathogens

Introduction

Entyloma contains more than 170 species of dicotyledon-parasitizing smut fungi, which are found on all continents except Antarctica (Vánky 2012). The phylogenetic relationships of *Entyloma* species based on ITS rDNA were established by Begerow et al. (2002), who showed that *Entyloma* evolution has been driven by its hosts. In a more recent study, both ITS sequence analysis and morphological data were used to delimit *Entyloma* spp. on *Eryngium* (Savchenko et al. 2014). The integration of molecular phylogenetic analyses, host plant taxonomy, and morphology provides a natural classification of these fungi.

Table 1. GenBank sequences used in this study

Species	Genbank No.	Reference
Entyloma atlanticum	AY081018	Begerow et al., 2002
E. arnicale	AY854965	Boekhout et al., 2006
E. australe	AY081019	Begerow et al., 2002
E. bidentis	AY081020	Begerow et al., 2002
E. browalliae	AY081021	Begerow et al., 2002
E. calceolariae	AY081022	Begerow et al., 2002
E. calendulae	AY081023	Begerow et al., 2002
E. carmeli	KF310892, KF310895	Savchenko et al., 2014
E. chrysosplenii	AY081024	Begerow et al., 2002
E. comaclinii	AY081025	Begerow et al., 2002
E. compositarum	AY854966	Boekhout et al., 2006
E. corydalis	AY081027	Begerow et al., 2002
E. costaricense	AY081028	Begerow et al., 2002
E. doebbeleri	AY081032	Begerow et al., 2002
E. eryngii	AY854972, AY081033, KF310896, KF310897	Begerow et al., 2002; Boekhout et al., 2006; Savchenko et al., 2014
E. eryngii-cretici	KF310894, KF310895	Savchenko et al., 2014
E. eryngii-plani	AY081034	Begerow et al., 2002
E. eschscholziae	KC456226	Genbank, unpublished
E. fergussonii	AY854971	Boekhout et al., 2006
E. ficariae	AY081035, JQ586199	Begerow et al., 2002; Savchenko et al. 2012
E. fuscum	AY081036	Begerow et al., 2002
E. gaillardianum	AY081037	Begerow et al., 2002
E. guaraniticum	AY081038	Begerow et al., 2002
E. hieracii	AY081039	Begerow et al., 2002
E. holwayi	AY081040	Begerow et al., 2002
E. linariae	AY081041	Begerow et al., 2002
E. lobeliae	AY081042	Begerow et al., 2002
E. madiae	AY081043	Begerow et al., 2002
E. magocsyanum	KF310891	Savchenko et al., 2014
E. matricariae	AY081044	Begerow et al., 2002
E. microsporum	AY081045	Begerow et al., 2002
E. polysporum	AY081046	Begerow et al., 2002
E. serotinum	AY081048	Begerow et al., 2002
E. ranunculi-repentis	AY081047	Begerow et al., 2002

Apiaceae harbors ca. twenty *Entyloma* species (Begerow et al. 2002; Vánky 2012), five of which are known on hosts of subfamily *Apioideae: E. bupleuri* Lindr. on *Bupleurum* (Liro 1904), *E. helosciadii* Magnus on *Helosciadium* (Magnus 1882), *E. kundmanniae* Malençon & Massenot on *Kundmannia* (Guyot et al. 1969), *E. magocsyanum* Bubák on *Tordylium* (Bubák 1907), and *E. pastinacae* Jaap on *Pastinaca* (Jaap 1916). Both *Entyloma flavum* Cif. on *Berula* and *Sium* (Ciferri 1924) and *E. oenanthes* Maire on *Oenanthe* (Maire et al. 1901) are considered conspecific with *E. helosciadii* s.l. (Reid 1957; Vánky 1994). Species delimitation of *Entyloma* on *Apioideae* is based primarily on host plant taxonomy and secondarily on minor differences in sori and spore morphologies. The analysis by Savchenko et al. (2014) clustered together *Entyloma* species on *Apiaceae*. That analysis was based mostly on the smut parasites of *Eryngium* (subfamily *Saniculoideae*) and included only one species (*Entyloma magocsyanum*) that parasitized a host from subfamily *Apioideae*.

During a 2011 survey on the diversity of smut fungi in Israel, a few infected *Scandix verna* plants were collected in two localities in northern Israel. Previously, no *Entyloma* species had been recorded on *Scandix*. The present study aims to resolve the specific status of the smut on *S. verna* through morphological and ITS sequence analyses and to determine its phylogenetic affinities within *Entyloma*.

Materials & methods

Herbarium samples are deposited in Haifa University Herbarium, Haifa, Israel (HAI); Washington State University Herbarium, Pullman, U.S.A. (WSP); and United States National Fungus Collection, Beltsville, U.S.A. (BPI). We were not able to include an *Entyloma kundmanniae* specimen in our analysis, so the information given on that species is based on Guyot et al. (1969) and Vánky (2012). Our hologenetype concept follows Chakrabarty (2010).

Sorus and spore characteristics were studied using dried herbarium material. Specimens were examined by light microscopy (LM). Pictures of sori were taken with a Canon Power Shot G10 camera. For LM, small pieces of leaf tissue with sori were mounted in 90% lactic acid on a microscope slide to which several drops of distilled water were added after which the slide was heated to the boiling point and cooled. The softened sori were cut in long pieces and squashed with a lancet, covered with a cover glass, gently heated to boiling point to eliminate air bubbles, and then examined under a Carl Zeiss Axiostar™ light microscope at 1000× magnification. LM photographs were taken with a Canon Power Shot G10 camera. At least 50 spores were measured from each collection, and the variation is presented as a range, with extreme values given in parentheses. Mean and standard deviations (SD) calculated from spores measured in all specimens are provided after the spore size ranges.

For SEM studies, spores were attached to metal stubs by double-sided adhesive tape and coated with gold. Spore surface ornamentation was observed at 15 kV and

photographed with a JEOL JSM-6700F scanning electron microscope with a working distance of ca. 12–13 mm.

Most sequences were obtained from GenBank (Table 1). Three additional sequences were generated for this analysis (Table 2). Genomic DNA was isolated from sori removed from herbarium specimens and lysed in 1.5 mL tubes for 1 min using FastPrep®24. Tubes were incubated in a water bath for 5 hours at 55 °C, and DNA extracted using DNeasy Plant Mini Kit (QIAGEN) following the manufacturer's instructions.

All amplifications were performed in 20 µl aliquots on an Applied Biosystems® GeneAmp 9700 thermal cycler. ITS5 or ITS1 were used as the forward primer and ITS4 as the reverse primer (White et al. 1990).

Standard cycling parameters with an annealing temperature of 57 °C were used for amplification. PCR products were purified with USB ExoSAP-IT according to the manufacturer's instructions and amplified with respective forward and reverse PCR primers with the BigDye® Terminator v3.1 Cycle Sequencing Kit. Those products were sequenced on an ABI PRISM® 3100 Genetic Analyzer.

The data were initially assembled and edited in Sequencher 4.5 for Windows, and the consensus sequences were aligned using MAFFTv6 (Katoh et al. 2002; Katoh & Toh 2008) under the default settings. Ambiguously aligned regions were recorded using GBlocks 091.b (Castresana 2000) with these options: minimum number of sequences for a conserved position to 29, minimum number of sequences for a flank position to 29, maximum number of contiguous non-conserved positions to 8, minimum length of a block to 5 and allowed gap positions to with half, and excluded from the analyses.

PAUPv40b10 was used for parsimony analysis, with trees inferred using the heuristic search option with 1000 random sequence additions. Maxtrees were unlimited, branches of zero length were collapsed, and all multiple parsimonious trees were saved. Descriptive tree statistics for parsimony (Tree Length [TL], Consistency Index [CI], Retention Index [RI], Relative Consistency Index [RC], and Homoplasy Index [HI] were calculated for trees generated under different optimality criteria. Bootstrap analysis (BS) (Hill & Bull 1993) was based on 1000 replications.

Bayesian analysis using a Monte Carlo Markov Chain (MCMC) technique was implemented using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). Four MCMC chains were run simultaneously, starting from random trees, for 1,000,000 generations. Trees were sampled every 100th generation for a total of 10,000 trees. The first 2000 trees were discarded as the burn-in phase of each analysis. Posterior probabilities (PP) (Rannala & Yang 1996) were determined from a majority-rule consensus tree generated with the remaining 8,000 trees. This analysis was done four times starting from different random trees to ensure that trees from the same tree space were being sampled during each analysis.

Trees were rooted using *Entyloma atlanticum* as in Begerow et al. (2002).

Results

Morphological analyses

All examined *Entyloma* specimens (Table 2) produced sori in the leaves of their hosts. The color of sori ranged from white in young sori to pale yellowish

Table 2. *Entyloma* specimens examined in this study

Species	Host	Voucher	Origin	Collector/ Year	GenBank
E. bupleuri	*Bupleurum subovatum*	HUJ	Israel	Chabelska/1942	
E. helosciadii	*Apium nodiflorum*	BPI 175486	Ireland	O'Connor/1945	
	Apium nodiflorum*	BPI 175487	Algeria	Maire/1916	
	Berula angustifolia	WSP 34402	Germany	Unknown/1900	
	Helosciadium repens	WSP 34403	Algeria	Unknown/1904	
	Oenanthe silaifolia	WSP 70797	Romania	Vánky1965	
E. magocsyanum	*Tordylium cordatum*	HAI 4625	Israel	Savchenko/2011	KF310891
	Tordylium maximum	WSP 66423	Romania	Vánky/1966	
	Tordylium maximum	BPI 175835	Romania	Bubák/1905	
	Tordylium maximum	BPI 175836	Romania	Bubák/1905	
E. pastinacae	*Pastinaca opaca*	BPI 176163	Romania	Săvulescu & Rayss/1930	
E. scandicis	*Scandix verna*	HAI 4799	Israel	Savchenko/2011	KF447773
	Scandix verna	HAI 4805	Israel	Savchenko/2011	KF447774
	Scandix verna	HAI 4806	Israel	Savchenko/2011	KF447775

*as *Helosciadium*

brown in old sori. The spores in all specimens were solitary, in chains, or in groups (but never crowded) and variable in size within a collection. Asexual states were present in all species except *E. bupleuri*. Spore sizes were similar in all species, but spore wall thicknesses varied from 0.5–1 µm in *Entyloma magocsyanum* to 1–3 µm in *E. helosciadii*. The color of sori varied from brown in *Entyloma bupleuri* to white in *E. scandicis* (and sometimes *E. helosciadii*).

Phylogenetic analyses

The ITS alignment included 48 sequences (including three sequences of *Entyloma* from *Scandix verna*) comprising 655 characters (including gaps). Parsimony analysis revealed that 477 characters are constant and of the variable characters 77 are parsimony-uninformative and 101 are parsimony informative. The parsimony analysis yielded 45 equally parsimonious trees, and the strict consensus tree of all equally parsimonious trees was used.

The different runs of Bayesian phylogenetic analyses yielded consistent topologies. We present the consensus tree of one run of Bayesian phylogenetic analyses to illustrate our results (Fig. 1). All analyses clustered together (with high support: PP = 1.0; BS = 93) the sequences of the smut on *Scandix verna*, which formed the sister lineage to *Entyloma magocsyanum*. The sequences of the *Scandix verna* smut from Carmel and Lower Galilee regions were identical to one another.

The phylogenetic relationships among species of *Entyloma* on *Apiaceae* inferred here are congruent with those in the study on *Entyloma* on *Eryngium* (Savchenko et al. 2014).

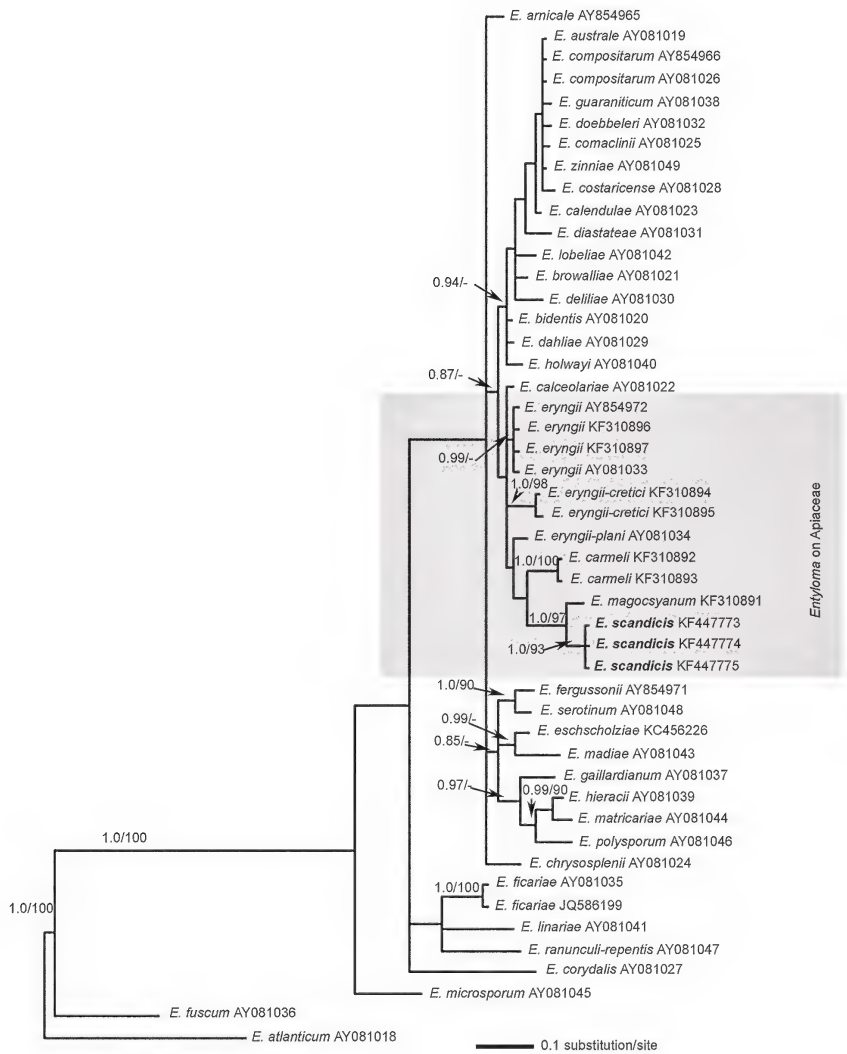

Fig. 1 Bayesian inference of phylogenetic relationships of *Entyloma* spp. resulting from the analysis of ITS nucleotide sequence data. Numbers on branches are estimates for PPs >0.8 from Bayesian inference (before /) and BS values >70 (after /). The tree was rooted using *Entyloma atlanticum*.

Taxonomy

Entyloma scandicis K.G. Savchenko, Carris, Castl., Heluta, Wasser & Nevo **sp. nov.** FIGURE 2

MYCOBANK MB 811390

Differs from *Entyloma helosciadii* by its paler spores and its specialization on *Scandix*.

TYPE: Israel. Lower Galilee, 2 km E of Kiryat Ata, 32°82′26″N 35°13′90″E, ca. 350 m asl, on *Scandix verna* O. Cohen, 15.04.2011, leg. K.G. Savchenko (**Holotype**, HAI 4799: GenBank KF447773).

ETYMOLOGY: The name of the species refers to the host plant genus, *Scandix*.

Sori in leaves, in round or elongate to polyangular amphigenous flat scattered or sometimes gregarious spots, initially white, later pale yellowish brown, 0.2–1 mm diameter or larger when confluent. Spores singular or in small irregular groups, sometimes in chains, globose, subglobose, ovoid, ellipsoidal to slightly irregular, (7) 8–11 × 9.5–15 (17) μm diameter [av. ± SD, 9.8 ± 1.6 × 12.6 ± 2.5 μm], subhyaline. Spore wall uniform, 1.5–2 (2.5) μm thick, hyaline to subhyaline, smooth. Anamorphic state present as fascicles of conidia and conidiophores, forming the amphigenous, white cover.

ADDITIONAL SPECIMENS EXAMINED: **ISRAEL, HAIFA**, Mount Carmel, University of Haifa campus, 32°75′83″N 35°02′32″E, ca. 430 m a.s.l., on *Scandix verna*, 11.04.2011, leg. K.G. Savchenko (HAI 4805; GenBank KF447774); Mount Carmel, Carmel National Park, 32°75′86″N 35°02′41″E, ca. 440 m asl, on *Scandix verna*, 30.03.2011, leg. K.G. Savchenko (HAI 4806; GenBank KF447775).

COMMENTS – The infected plants were found in March and April in two localities in northern Israel in different habitats. At Mount Carmel, *Entyloma scandicis* occurred on host plants in a shaded forest community under *Pinus halepensis*. In the Lower Galilee location, the fungus occurred on host plants in an open forest in glades between *Cupressus sempervirens*. Both locations are typical for Israel's Mediterranean floristic zone (Zohary 1973). Certainly, *Entyloma scandicis* is a Mediterranean forest species, hitherto not known outside of northern Israel. *Entyloma scandicis* was not found in steppe and semi-desert areas within the area of host distribution despite examination of thousands of host plants.

Discussion

Scandix is a monophyletic genus of the tribe *Scandiceae* (Downie et al. 1998, 2000; Lee & Downie 2000; Spalik et al. 2001) of which no members were previously known to host smut fungi (Vánky 2012). Our molecular phylogenetic analyses and morphological data have helped to resolve the systematic position of *Entyloma* on *Scandix verna*.

Entyloma scandicis can be distinguished from *E. bupleuri* by the sorus color (yellowish white in *E. scandicis,* brown in *E. bupleuri*) and the presence of an *Entylomella*-like asexual stage in *E. scandicis.* The new species can be separated from *Entyloma kundmanniae, E. magocsyanum,* and *E. pastinacae* by thicker spore walls (1.5–2.5 µm in *E. scandicis* vs. ≤1.5 µm in the other three). In several aspects, such as in spore dimensions, *Entyloma scandicis* resembles *E. helosciadii,* which differs in its pale to dark yellow spores.

ITS phylogenetic analysis recovered all three *Entyloma* specimens on *Scandix verna* in a strongly supported monophyletic group forming an independent lineage (Fig. 1). Morphologically there is no difference between the Mt Carmel and Lower Galilee specimens. As in the molecular phylogenetic analysis of a smaller sampling of *Entyloma* species on *Apiaceae* (Savchenko et al. 2014), our analysis clustered together all *Entyloma* species on *Apiaceae* within the /apiaceae clade and in sharing a common ancestor with other *Entyloma* groups on asterid hosts. Interestingly, *E. calceolariae* Lagerh., a parasite on species in the family *Calceolariaceae*, also clustered within the /apiaceae clade (Fig. 1).

So far, only two species of *Entyloma* on *Apioideae—E. magocsyanum* and *E. scandicis*—have undergone molecular phylogenetic analyses (Savchenko et al. 2014). It should be noted that molecular-oriented studies have implied significant radiation for some *Entyloma* species (Savchenko et al. 2014; Vánky & Lutz 2010), which supports the hypothesis that *Entyloma* species have relatively narrow host ranges restricted to a single genus or, in some cases, a single host species (Savchenko et al. 2014; Savile 1947; Vánky 1994, 2009). Additional sequencing is needed to resolve the taxonomy of *Entyloma helosciadii,* a complex of three species known to infect hosts from four different genera in the tribe *Oenantheae* (Reid 1957; Vánky 1994). Another taxonomic problem is the possible conspecificity of the pathogenic *Entyloma magocsyanum* and *E. pastinacae*, both with hosts in tribe *Tordyleae* and with no diagnostic morphological differences.

This study shows that genetic divergence, a different host tribe, and different spore and soral morphologies support *Entyloma scandicis* on *Scandix verna* as a new species. This species fills a gap in the distribution of *Entyloma* on representatives of the tribe *Scandiceae*. Future molecular phylogenetic studies combined with morphological methods may well reveal higher diversity within the species of *Entyloma* on *Apioideae*.

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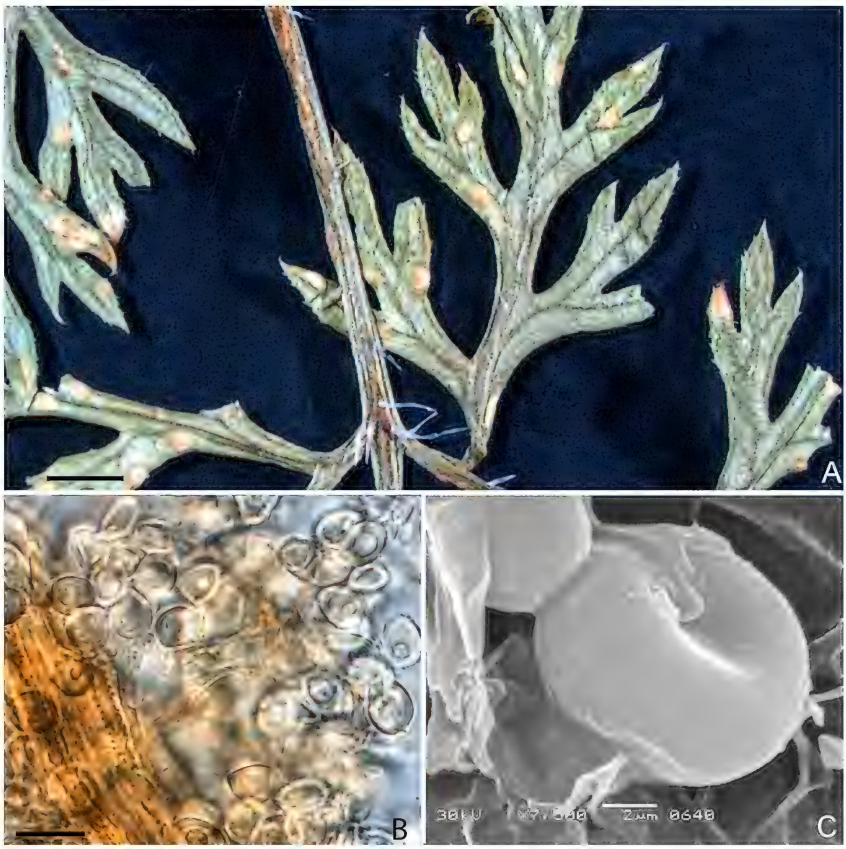

Fig. 2 *Entyloma scandicis* (holotype, HAI 4799): A. sori in leaves of *Scandix verna*; B. spores (LM); C. spores (SEM). Scale bars: A = 2 mm, B = 20 µm, C = 2 µm.

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Myxomycetes of Chihuahua (México) 4. Central plains of the Chihuahuan desert

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Abstract—Collections of myxomycetes gathered in field and cultivated in moist chamber cultures from samples collected in 10 localities in the central plains of the Chihuahuan desert were studied. Of the 37 species identified, eight (*Badhamia spinispora, B. verrucospora, Comatricha ellae, Didymium tehuacanense, Echinostelium paucifilum, Lycogala exiguum, Physarum apiculosporum*, and *P. spectabile*) represent new records for Chihuahua. *Badhamia verrucospora, Echinostelium paucifilum*, and *Physarum apiculosporum* are recorded for the first time from Mexico.

Key words—*Amoebozoa*, chorology, myxobiota, *Myxomycota*, SEM, slime moulds, taxonomy

Introduction

With a total area of 507,000 km^2, the Chihuahuan desert is the largest in North America and extends from New Mexico and Texas in the United States to north of Oaxaca in Mexico. It ranges in elevation between 1000–2000 m and has an annual average temperature of 16–18°C. Temperatures lower than 0°C have been recorded in some northern regions during winter. In summer, the most important precipitation occurs in the form of monsoons, although not exceeding 400 mm annually.

The vegetation of the Chihuahuan desert consists mainly of microphyllous scrubs, with *Larrea tridentata* (DC.) Coville (*Zygophyllaceae*), *Fouquieria*

Table 1. Studied localities and vegetation types

Locality	Latitude N	Longitude W	Elevation	Vegetation
Town Juárez				
1. Samalayuca	31°13′53.1″	106°30′36.8″	1120 m	SDV
Town Ascensión				
2. Ascención	31°36′36.0″	106°47′03.0″	1300 m	SDV
Town Guadalupe				
3. El Cuervo	31°07′54.7″	105°42′47.8″	1100 m	MDS
Town Ahumada				
4. Candelaria	31°07′32.0″	106°29′48.0″	1280 m	MDS
Town Ojinaga				
5. La Mula	29°14′24.0″	104°26′09.0″	1380 m	MDS
6. Puente Pegüis II	29°32′28.9″	104°42′51.7″	770 m	MDS
Town Manuel Benavides				
7. Camino a San Antonio, km 2	29°05′39.1″	103°52′38.0″	1065 m	MDS
Town Delicias				
8. Parque Industrial	28°06′27.2″	105°24′53.9″	1160 m	MDS
Town Camargo				
9. Carret. a Ojinaga Km 42	27°51′07.1″	104°44′04.7″	1265 m	MDS
Town Jiménez				
10. Establo El Parral	27°24′53.3″	104°53′37.2″	1370 m	MDS

Vegetation: MDS = microphyllous desert scrub; SDV = sandy desert vegetation

splendens Engelm. (*Fouquieriaceae*), *Prosopis glandulosa* Torr. (*Leguminosae*), and several species of *Yucca* and *Agave* (*Asparagaceae*) among the main dominants. Especially prominent are a great diversity of small and medium-sized cacti (*Cactaceae*), including species of *Coryphantha, Cylindropuntia*, *Echinocereus*, *Mammillaria*, and *Opuntia* (Rzedowski 2006).

Despite its considerable biological diversity, only a few studies of its myxobiota have been conducted, most of which have focused on pine-oak forest ecosystems (Lizárraga et al. 2004a, 2005a,b, 2006; Moreno et al. 2006a,b; Salazar-Márquez et al. 2013). Other works on similar desert areas have been carried out mostly in central and southern Mexico (Mosquera et al. 2003, Lado et al. 2007, Wrigley de Basanta et al. 2008, Estrada-Torres et al. 2009). In contrast, few references exist for microphyllous desert scrub (Lizárraga et al. 2003, 2005a,b; Salazar-Márquez et al. 2014) for the more northern Chihuahuan desert.

During the past decade, the catalog of myxomycetes of the Chihuahua has been increased from 119 (Lizárraga et al. 2015) to 127 taxa (125 species and 2

varieties) due to research by Moreno et al. (2007), Lizárraga et al. (2015), and Salazar-Márquez et al. (2013, 2014).

Materials & methods

The survey was carried out in the central plains of the Chihuahuan desert. Ten localities with two types of vegetation were sampled seasonally from spring to winter 2009 (Table 1); each sampling effort took a week. The studied specimens were collected in the field or obtained in moist chamber cultures. Specimens are deposited in the collection of fungi of the Universidad Estatal de Sonora, Hermosillo, Mexico (UES), with a duplicate of some samples conserved in the herbarium of the Life Sciences Department (Botany), University of Alcalá, Madrid, Spain (AH). Permanent slides were prepared with Hoyer's liquid for microscopic study. Collectors names are abbreviated as follows: A. Gutiérrez (AG), M. Lizárraga (ML), D. López (DL), D. Sáenz (DS), C. Salazar (CS), and A. Sánchez (AS).

Spores (including surface structures such as spines or warts) were measured with an oil immersion lens. Light microscopy (LM) was carried out with a Nikon microscope equipped with an automatic photographic system. SEM micrographs were produced with a Zeiss DSM-950 microscope. For ultramicroscopic studies, material was rehydrated in concentrated (28–30%) ammonium hydroxide for 30 min, dehydrated in aqueous ethanol (70%) for 30 min, fixed for 2 h in pure ethylene glycol dimethyl ether (= 1,2–dimethoxymethane), and finally immersed in pure acetone for at least 2 h. This was followed by critical point drying and sputtering with gold-palladium. This technique allows the use of very little material (i.e., part of a single sporocarp or sometimes no more than a few spores).

Taxonomy

Arcyria cinerea (Bull.) Pers., Syn. Meth. Fung.: 184 (1801).

Specimens examined: **MEXICO, Chihuahua:** Loc. 1, *Yucca elata* Engelm., 24 July 2009, leg. ML, CS, DL, DS & AG, obtained from moist chamber 12 August 2009 (UES 8250); rat dung, 23 October 2009, leg. ML, CS, AS, AG & DL, obtained from moist chamber 9 December 2009 (UES 8380); Loc. 3, *Tamarix chinensis* Lour. (*Tamaricaceae*), 20 May 2009, leg. ML, CS & DL, obtained from moist chamber 30 June 2009 (UES 7977); Loc. 6, *Agave lecheguilla* Torr., 23 March 2009, leg. ML & CS, obtained from moist chamber 20 April 2009 (UES 7561); *Euphorbia antisyphilitica* Zucc. (*Euphorbiaceae*), 25 May 2009, leg. ML, CS & DL, obtained from moist chamber 29 May 2009 (UES 7974); obtained from moist chamber 3 June 2009 (UES 7975, AH 45575). Loc. 10, rabbit dung, 25 July 2009, leg. ML, CS, DL, DS & AG, obtained from moist chamber 5 August 2009 (UES 8255).

Observations — *Arcyria cinerea* was obtained in moist chamber on different substrates such as rat and rabbit dung. Its coprophilous habit has been noted by Eliasson & Lundqvist (1979), Eliasson & Keller (1999), and Krug et al. (2004). Previously cited for Chihuahua by Lizárraga et al. (2003).

Arcyria denudata (L.) Wettst., Verarb. Pilzfl. Steiermark 1: 9 (1885).

Specimen examined: **MEXICO, Chihuahua:** Loc. 6, *Yucca* sp. 23 March 2009, leg. ML & CS (UES 7562, AH 45576).

Observations — Previously cited for Chihuahua by Lizárraga et al. (2003).

Badhamia affinis Rostaf., Sluzowce Monogr.: 143 (1874).

Specimen examined: **MEXICO, Chihuahua:** Loc. 7, *Prosopis glandulosa*, 26 July 2009, leg. ML, CS, AG, DL & DS (UES 8262, AH 45577).

Observations — See Moreno & Oltra (2010) for a detailed study of *Badhamia affinis,* including photomicrographs of the spore ornamentation.

Badhamia gracilis (T. Macbr.) T. Macbr., Myxomycetes: 35 (1934).

Diagnostic characters include dark polygonal (12–14 µm diam.) warted spores and a badhamioid capillitium.

Specimens examined: **MEXICO, Chihuahua:** Loc. 1, rabbit dung, 22 May 2009, leg. ML, CS & DL, obtained from moist chamber 30 June 2009 (UES 8085); rat dung, obtained from moist chamber 30 June 2009 (UES 8087); Loc. 2, *Yucca elata*, 7 February 2009, leg. ML, CS & DS (UES 7690); obtained from moist chamber 25 February 2009 (UES 7691); 22 May 2009, leg. ML, CS & DL (UES 7982); rat dung, obtained from moist chamber 19 March 2009 (UES 7688); cow dung, 23 July 2009, leg. ML, CS, DL, AG & DS, obtained from moist chamber 8 September 2009 (UES 8362); Loc. 3, *Opuntia macrocentra* Engelm., 20 May 2009, leg. ML, CS & DL (UES 7978, 7979, 7980); 23 July 2009, leg. ML, CS, DL, DS & AG (UES 8266); obtained from moist chamber 3 August 2009 (UES 8268); obtained from moist chamber 8 August 2009 (UES 8267); obtained from moist chamber 10 August 2009 (UES 8220); obtained from moist chamber 12 August 2009 (UES 8239); Loc. 4, *Opuntia macrocentra*, 14 February 2009, leg. ML, CS & DS (UES 7694); obtained from moist chamber 26 February 2009 (UES 7695); obtained from moist chamber 2 March 2009 (UES 7696); Loc. 5, *Opuntia* sp., 25 May 2009, leg. ML, CS & DL, obtained from moist chamber (UES 7992); 25 October 2009, leg. ML, CS, AS, AG & DL (UES 8379); obtained from moist chamber 6 November 2009 (UES 8384); obtained from moist chamber 14 November 2009 (UES 8385); obtained from moist chamber 27 November 2009 (UES 8386, 8387); Loc. 6, *Yucca* sp., 23 March 2009, leg. ML & CS, obtained from moist chamber 20 April 2009 (UES 7706, 7707); *Agave lecheguilla*, 25 May 2009, leg. ML, CS & DL (UES 7988); Loc. 7, *Echinocereus enneacanthus* Engelm., 22 March 2009, leg. ML & CS, obtained from moist chamber 14 April 2009 (UES 7702); *Yucca* sp., obtained from moist chamber 20 April 2009 (UES 7703); Loc. 8, *Opuntia* sp., 23 March 2009, leg. ML & CS, obtained from moist chamber 13 April 2009 (UES 7709); Loc. 9, *Opuntia macrocentra,* 25 July 2009, leg. ML, CS, DL, DS & AG, obtained from moist chamber 22 August 2009 (UES 8313); obtained from moist chamber 29 August 2009 (UES 8316, AH 45578).

Observations — *Badhamia gracilis,* which is succulenticolous, has been the most abundant species in both the field and moist chamber cultures in most studied localities. We cite only a limited number of samples for each locality, but much more material was deposited in the herbarium. Study of the spore

ornamentation under SEM by Moreno & Oltra (2010) demonstrated the morphological separation of this species from *Badhamia melanospora* Speg.

Badhamia gracilis was reported on dung by Krug et al. (2004); in this study, it was observed in rabbit, rat and cow dung.

Previously cited for Chihuahua by Lizárraga et al. (2003) and Salazar-Márquez et al. (2014).

****Badhamia spinispora*** (Eliasson & N. Lundq.) H.W. Keller & Schokn., Mycologia 81: 632 (1989). Figs 1, 2

Fructifications sessile subglobose sporocarps to short plasmodiocarps, white to grayish, growing solitary or grouped. Capillitium varying from physaroid to badhamioid, consisting of irregular filaments and calcareous nodules. Spores black in mass, light violaceous under LM, globose, subglobose to oval, 10–12 µm diam., ornamented by obvious spines.

Specimens examined: **MEXICO, Chihuahua:** Loc. 1, rat dung, 22 May 2009, leg. ML, CS & DL, obtained from moist chamber 25 June 2009 (UES 7994); Loc. 2, cow dung, 26 October 2009, leg. ML, CS, AS, AG & DL, obtained from moist chamber 9 December 2009 (UES 8361, AH 45555).

Observations — *Badhamia spinispora* is a rare species, generally with a coprophilous habit. The differences between it and *Badhamia verrucospora* and *Physarum apiculosporum* are detailed in the observations under those species.

Previously cited for Sonora by Esqueda et al. (2013).

*****Badhamia verrucospora*** G. Moreno, D.W. Mitch. & Novozh., Bol. Soc. Micol. Madrid 35: 98 (2011). Figs 3, 4

Fructifications subglobose sporocarps to short plasmodiocarps, in groups, white, sessile, 0.2–0.5 × 0.2–1 mm. Peridium double, calcareous, with irregular dehiscence. Capillitium scarce, consisting of filaments connected by irregular calcareous nodules. Spores (15–)17–18 × 10–12 µm diam., black in mass, purple under LM, subglobose to oval, a broad part of its surface is ornamented with clearly marked warts, the rest of the spore has a thinner wall and smaller warts, sometimes with ornamentation almost lacking.

Specimens examined: **MEXICO, Chihuahua:** Loc. 1, cow dung, 14 February 2009, leg. ML, CS & DS, obtained from moist chamber 7 March 2009 (UES 7644, AH 45556); 24 July 2009, leg. ML, CS, DL, DS & AG (UES 8269); obtained from moist chamber 25 August 2009 (UES 8370); obtained from moist chamber 1 September 2009 (UES 8317); obtained from moist chamber 8 September 2009 (UES 8371); Loc. 2, cow dung, 7 February 2009, leg. ML, CS & DS, obtained from moist chamber 9 March 2009 (UES 7641); obtained from moist chamber 18 March 2009 (UES 7642, AH 45578); obtained from moist chamber 31 March 2009 (UES 7643); rabbit dung, obtained from moist chamber 1 September 2009 (UES 8318); 22 October 2009, leg. ML, CS, AS, AG & DL, obtained from moist chamber 9 December 2009 (UES 8517, 8518); rabbit dung, 23 July

2009, leg. ML, CS, DL, DS & AG, obtained from moist chamber 8 September 2009 (UES 8365); Loc. 3, cow dung, 23 July 2009, leg. ML, CS, DL, DS & AG, obtained from moist chamber 8 September 2009 (UES 8376); Loc. 4, cow dung, 14 February 2009, leg. ML, CS & DS, obtained from moist chamber 26 March 2009 (UES 7645); Loc. 5, horse dung, 24 May 2009, leg. ML, CS & DL, obtained from moist chamber 30 June 2009 (UES 7995); Loc. 7, cow dung, 22 October 2009, leg. ML, CS, AS, AG & DL, obtained from moist chamber 9 December 2009 (UES 8530).

Observations — *Badhamia spinispora* (Eliasson & Lundqvist 1979; as *Physarum spinisporum*), is morphologically similar to *P. apiculosporum* and *B. verrucospora*. It is characterized by its globose to broadly oval spores with sharp spines, while *P. apiculosporum* has oval to ellipsoidal spores that are smooth and with two apiculi. *Badhamia verrucospora* spores are similar to those of *Physarum apiculosporum* (oval to ellipsoid with two apiculi) but ornamented by abundant small warts over half the spore surface (Moreno et al. 2011). *Badhamia rhytidosperma* H.W. Keller & Schokn., which resembles the three previous species but with reticulate spores, has not yet been reported from Mexico, according to the consulted literature.

Comatricha elegans (Racib.) G. Lister, Guide Brit. Mycetozoa, 3rd Ed.: 31 (1909). Figs 9–11

Sporocarps small (0.5–1 mm tall); columella branched; stipe fibrous; the capillitium forming an external link with well-defined more or less continuous meshes; spores 9–10 µm diam., warted.

Specimen examined: **MEXICO, Chihuahua:** Loc. 3, *Tamarix chinensis*, 20 May 2009, leg. ML, CS & DL, obtained from moist chamber 29 May 2009 (UES 8065, AH 45557).

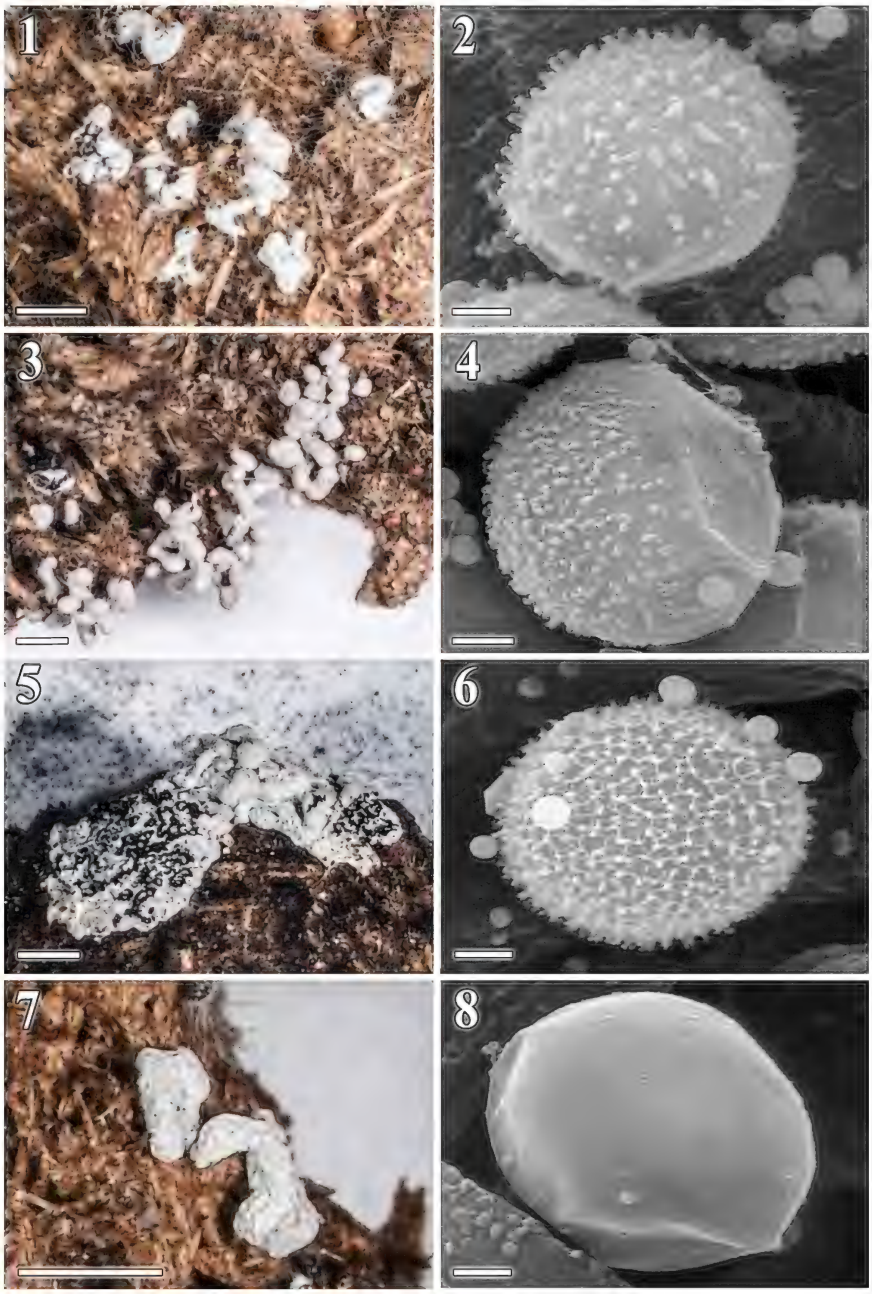
Observations — A SEM study of *Comatricha elegans* was carried out by Lizárraga et al. (2005a).

Poorly known in Mexico (Moreno et al. 2007), *C. elegans* has been previously recorded for Chihuahua by Lizárraga et al. (2005b) and Salazar-Márquez et al. (2014).

****Comatricha ellae*** Härk., Karstenia 18: 23 (1978). Fig. 12

Sporocarps small (0.5–1 mm tall); sporotheca 0.2–0.4 mm diam.; capillitium with well-defined meshes mostly into stipe junction; columella percurrent, spores 9–10 µm diam.

Figs 1–8. ***Badhamia spinispora*** (AH 45555). 1. Plasmodiocarps. 2. Spore spinulose (SEM). ***Badhamia verrucospora*** (AH 45556). 3. Plasmodiocarps. 4. Spore verrucose (SEM). ***Fuligo cinerea*** (AH 45574). 5. Plasmodiocarps. 6. Spore reticulate (SEM). ***Physarum apiculosporum*** (AH 45566). 7. Plasmodiocarps. 8. Spore smooth (SEM). Scale bars: 1, 3, 5, 7 = 1 mm; 2, 4, 6, 8 = 2 µm.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Specimen examined: **MEXICO, Chihuahua:** Loc. 1, *Artemisia filifolia* Torr. (*Asteraceae*), 24 July 2009, leg. ML, CS, DL, DS & AG, obtained from moist chamber 5 August 2009 (UES 8307, AH 45558).

Observations — We observed this species frequently in moist chamber cultures on succulent plant crusts.

Comatricha ellae was cited previously only from Baja California Sur (Moreno et al. (2007).

Comatricha tenerrima (M.A. Curtis) G. Lister, Guide Brit. Mycetozoa, 4th Ed.: 39 (1919).

Characterized by the acuminate sporothecal apex, sinuous capillitium, and spores light violaceous under LM and 8–9 µm diam., warted.

Specimens examined: **MEXICO, Chihuahua:** Loc. 10, *Cylindropuntia* sp., 21 March 2009, leg. ML & CS, obtained from moist chamber 6 April 2009 (UES 7742, AH 45579); *Koeberlinia spinosa* Zucc. (*Koeberliniaceae*), 25 July 2009, leg. ML, CS, DL, DS & AG, obtained from moist chamber 10 August 2009 (UES 8229).

Observations —Salazar-Márquez et al. (2014) reported that SEM photographs show that the spore ornamentation in *C. tenerrima* is formed by joined bacula with a characteristic star-shaped morphology.

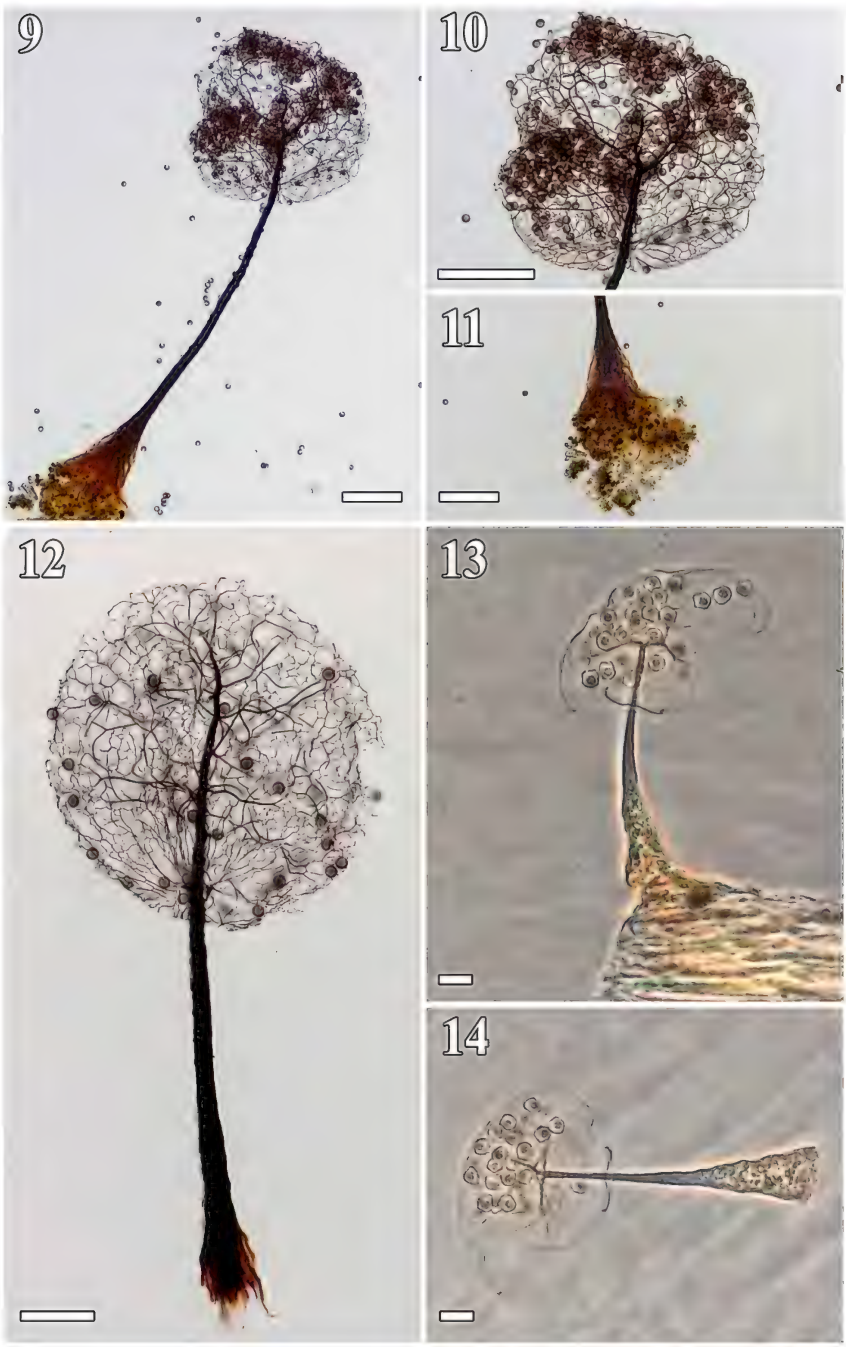
Previously cited for Chihuahua by Salazar-Márquez et al. (2014) and Lizárraga et al. (2005b).

Dictydiaethalium plumbeum (Schumach.) Rostaf. ex Lister, Monogr. Mycetozoa: 157 (1894).

Cultures in moist chambers showing considerable morphological variation; aethalia small (0.5–1 mm diam.), subglobose to pulvinate.

Specimens examined: **MEXICO, Chihuahua:** Loc. 1, *Prosopis glandulosa,* 22 May 2009, leg. ML, CS & DL, obtained from moist chamber 3 June 2009 (UES 8010); Loc. 3, *Larrea tridentata,* 24 January 2009, leg. ML, CS & DS, obtained from moist chamber 5 February 2009 (UES 7570); obtained from moist chamber 13 February 2009 (UES 7571); 22 October 2009, leg. ML, CS, AS, AG & DL, obtained from moist chamber 27 November 2009 (UES 8424); obtained from moist chamber 9 December 2009 (UES 8425); Loc. 4, 14 February 2009, leg. ML, CS & DS, obtained from moist chamber 28 February 2009 (UES 7572); obtained from moist chamber 1 April 2009 (UES 7573, AH 45580); Loc. 7, *Porlieria angustifolia* (Engelm.) A. Gray (*Zygophyllaceae*), 22 March 2009, leg. ML & CS, obtained from moist chamber 14 April 2009 (UES 7574); *Larrea tridentata*, 26 October 2009, leg. ML, CS, AS, AG & DL, obtained from moist chamber 21 November 2009 (UES 8421); obtained from moist chamber 27 November 2009 (UES 8423); *Prosopis glandulosa,* obtained from moist chamber 21 November 2009

Figs 9–14. ***Comatricha elegans*** (AH 45557). 9. Sporocarp. 10. Capillitium detail forming an external net and columella. 11. Stalk fibrous. ***Comatricha ellae*** (AH 45558). 12. Sporocarp. ***Echinostelium arboreum*** (AH 45560). 13, 14. Sporocarp detail, peridium persistent, membranous, collar, columella, capillitium, and spores. Scale bars: 9–12 = 100 µm; 13, 14 = 10 µm.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(UES 8422); Loc. 8, 23 March 2009, obtained from moist chamber 4 April 2009 (UES 7576); obtained from moist chamber 20 April 2009 (UES 7685); 24 October 2009, leg. ML, CS, AS, AG & DL, obtained from moist chamber 9 November 2009 (UES 8417); obtained from moist chamber 21 November 2009 (UES 8418); Loc. 9, *Acacia greggii* A. Gray (*Leguminosae*), leg. ML & CS, obtained from moist chamber 10 August 2009 (UES 8224); *Larrea tridentata,* obtained from moist chamber 22 August 2009 (UES 8326); 25 October 2009, leg. ML, CS, AS, AG & DL, obtained from moist chamber 14 December 2009 (UES 8415); obtained from moist chamber 9 December 2009 (UES 8416); Loc. 10, *Larrea tridentata,* 21 March 2009, obtained from moist chamber 8 April 2009 (UES 7575); *Prosopis glandulosa,* 22 May 2009, leg. ML, CS & DL, obtained from moist chamber 3 June 2009 (UES 8009); *Larrea tridentata,* 25 July 2009, leg. ML, CS, DL, DS & AG, obtained from moist chamber 8 August 2009 (UES 8287); obtained from moist chamber 12 August 2009 (UES 8254); obtained from moist chamber 15 August 2009 (UES 8325); 25 October 2009, leg. ML, CS, AS, AG & DL, obtained from moist chamber 6 November 2009 (UES 8419); obtained from moist chamber 9 November 2009 (UES 8420); obtained from moist chamber 14 December 2009 (UES 8427); obtained from moist chamber, 21 November 2009 (UES 8489).

Observations — We follow the broad species concept of Martin & Alexopoulos (1969), which accepts a widely variable aethalium size and color.

Previously cited for Chihuahua by Lizárraga et al. (2005a).

Didymium difforme (Pers.) Gray, Nat. Arr. Brit. Pl. 1: 571 (1821).

Spores warted, 13–15 µm diam.; sporotheca pulvinate with a crust-like peridium.

Specimen examined: **MEXICO, Chihuahua:** Loc. 4, rabbit dung, 14 February 2009, leg. ML, CS & DS, obtained from moist chamber 25 March 2009 (UES 7588).

Observations —Eliasson & Lundqvist (1979), Eliasson & Keller (1999) and Krug et al. (2004) reported this species from dung.

Didymium difforme is rarely cited from Mexico (Moreno et al. 2007). Previously reported for Chihuahua on rabbit dung (Salazar-Márquez et al. 2014).

Didymium dubium Rostaf., Sluzowce Monogr.: 143 (1874).

Specimens examined: **MEXICO, Chihuahua:** Loc. 1, *Prosopis glandulosa,* 14 February 2009, leg. ML, CS & DS, obtained from moist chamber 24 February 2009 (UES 7787); obtained from moist chamber 28 February 2009 (UES 7788, 7789, 7790); *Artemisia filifolia,* 14 February 2009, leg. ML, CS & DS, obtained from moist chamber 26 February 2009 (UES 7782); cow dung, obtained from moist chamber 26 February 2009 (UES 7783); obtained from moist chamber 28 February 2009 (UES 7784); obtained from moist chamber 31 March 2009 (UES 7785); rabbit dung, obtained from moist chamber 18 March 2009 (UES 7786); *Yucca* sp., 24 July 2009, leg. ML, CS, DL, DS & AG, obtained from moist chamber 12 August 2009 (UES 8334); Loc. 2, *Artemisia filifolia,* 7 February 2009, leg. ML, CS & DS, obtained from moist chamber 16 February 2009 (UES 7763); *Yucca elata,* obtained from moist chamber 16 February 2009 (UES 7776);

Prosopis glandulosa, 7 February 2009, leg. ML, CS & DS, obtained from moist chamber 21 February 2009 (UES 7767); rabbit dung, obtained from moist chamber 27 February 2009 (UES 7764); obtained from moist chamber 14 March 2009 (UES 7765); 23 July 2009, leg. ML, CS, DL, DS & AG, obtained from moist chamber 10 August 2009 (UES 8221); Loc. 3, *Prosopis glandulosa*, obtained from moist chamber 10 February 2009 (UES 7754); *Larrea tridentata*, 24 January 2009, leg. ML, CS & DS, obtained from moist chamber 3 February 2009 (UES 7745); *Tamarix chinensis*, 24 January 2009, leg. ML, CS & DS, obtained from moist chamber 23 February 2009 (UES 7761); *Fouquieria splendens*, 20 May 2009, leg. ML, CS & DL, obtained from moist chamber 3 June 2009 (UES 8011); Loc. 4, *Fouquieria splendens*, 14 February 2009, leg. ML, CS & DS, obtained from moist chamber 21 February 2009 (UES 7777); *Prosopis glandulosa*, 14 February 2009, leg. ML, CS & DS, obtained from moist chamber 2 March 2009 (UES 7770); *Yucca elata*, obtained from moist chamber 3 June 2009 (UES 8020); *Larrea tridentata*, 24 February 2009, leg. ML, CS, DL, DS & AG, obtained from moist chamber 10 August 2009 (UES 8223); Loc. 5, *Fouquieria splendens*, 22 March 2009, obtained from moist chamber 4 April 2009 (UES 7796); *Cylindropuntia imbricata* (Haw.) F.M. Knuth, 24 May 2009, leg. ML, CS & DL, obtained from moist chamber 29 May 2009 (UES 8032); *Prosopis glandulosa*, obtained from moist chamber 29 May 2009 (UES 8034); Loc. 6, *Prosopis glandulosa*, 23 March 2009, leg. ML & CS, obtained from moist chamber 4 April 2009 (UES 7806); *Fouquieria splendens*, obtained from moist chamber 6 April 2009 (UES 7804); *Agave lecheguilla*, 26 October 2009, disposed in moist chamber 2.XI.20009, fructifications 14 December 2009 (UES 8462); Loc. 7, *Agave lecheguilla*, 22 March 2009, leg. ML & CS, obtained from moist chamber 13 April 2009 (UES 7800); *Prosopis glandulosa*, 26.VII.09, leg. ML, CS, DL, DS & AG, obtained from moist chamber 8 August 2009 (UES 8284); *Larrea tridentata*, obtained from moist chamber 21 November 2009 (UES 8447); *Cylindropuntia imbricata*, obtained from moist chamber 12 August 2009 (UES 8245); Loc. 9, *Prosopis glandulosa*, 22 March 2009, leg. ML & CS, obtained from moist chamber 6 April 2009 (UES 7795); rabbit dung, 22 July 2009, leg. ML, CS, DL, AS & AG, obtained from moist chamber 15 August 2009 (UES 8335); Loc. 10, rabbit dung, 21 March 2009, leg. ML & CS, sporocarps obtained 13 April 2009 (UES 7794); *Acacia constricta* A. Gray (*Leguminosae*), 23 May 2009, leg. ML, CS & DL, obtained from moist chamber 3 June 2009 (UES 8025).

Observations — *Didymium dubium* exhibits great morphological variation in the sporocarps but can be recognized by spores 12–14 µm diam., with small bacula that form a characteristic reticle easily visible under SEM (Moreno et al. 2001). It was frequently collected in the studied area; thus, only samples of different localities and habitats are cited.

Previously recorded for Chihuahua by Lizárraga et al. (2003) and Salazar-Márquez et al. (2014).

Didymium mexicanum G. Moreno, Lizárraga & Illana, II Int. Congr. Syst. Ecol. Myxomycetes: 56 (1996).

= *Didymium umbilicatum* D. Wrigley, Lado & Estrada, Mycologia 100: 922 (2008).

Specimens examined: **MEXICO, Chihuahua:** Loc. 6, *Yucca* sp., 23 March 2009, leg. ML & CS (UES 7577); leg. ML, CS, DL, DS & AG, obtained from moist chamber 22 August 2009 (UES 8337); 26 October 2009, leg. ML, CS, AS, AG & DL, obtained from

moist chamber 9 November 2009 (UES 8455); *Agave lecheguilla,* obtained from moist chamber 27 November 2009 (UES 7578, 7579, 7580), obtained from moist chamber 4 April 2009 (UES 7581); obtained from moist chamber 10 April 2009 (UES 7582); obtained from moist chamber 14 April 2009 (UES 7802); obtained from moist chamber 20 April 2009 (UES 7803, 7812); obtained from moist chamber 1 May 2009 (UES 7583); 25 May 2009, leg. ML, CS & DL (UES 8027, 8028); obtained from moist chamber 25 June 2009 (UES 8030); obtained from moist chamber 30 June 2009 (UES 8031); 26 July 2009, leg. ML, CS, DL, DS & AG (UES 8286); 26 October 2009, leg. ML, CS, AS, AG & DL, obtained from moist chamber 27 November 2009 (UES 8459); obtained from moist chamber 18 December 2009 (UES 8463, 8465); obtained from moist chamber 5 January 2010 (UES 8466).

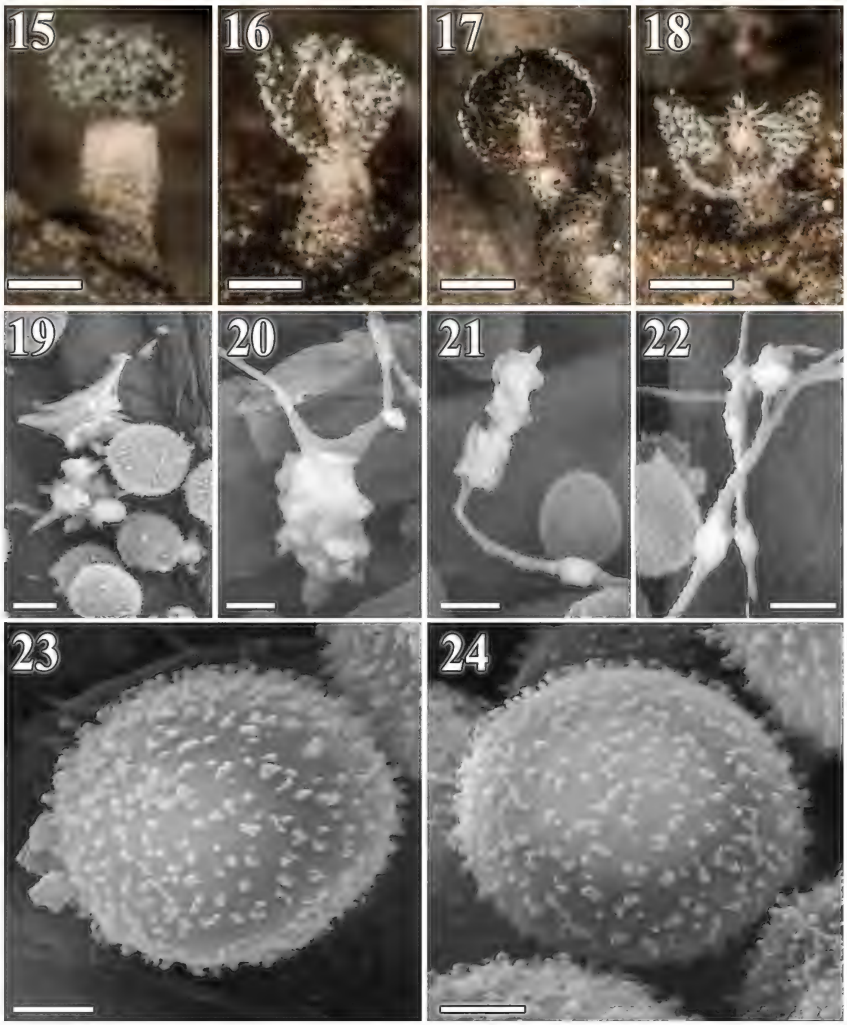
Observations — Moreno et al. (2012) included samples from Chihuahua in their recent macro- and microscopical (including SEM) examinations of *Didymium mexicanum* and *D. umbilicatum* that confirmed these species are synonyms.

In Mexico, this species is known from Baja California (Moreno et al. 1997, 2012; Lizárraga et al. 2004b), Chihuahua (Moreno et al. 2012; Salazar-Márquez et al. 2014), Hidalgo (Wrigley de Basanta et al. 2008, as *D. umbilicatum*), Oaxaca (Wrigley de Basanta et al. 2008, as *D. umbilicatum*; Estrada-Torres et al. 2009, as *D. umbilicatum*), Puebla (Wrigley de Basanta et al. 2008, as *D. umbilicatum*; Estrada-Torres et al. 2009, as *D. umbilicatum*), and Querétaro (Wrigley de Basanta et al. 2008, as *D. umbilicatum*). It has also been cited from Argentina (Lado et al. 2011), Guatemala (Wrigley de Basanta et al. 2008, as *D. umbilicatum*), and Russia (Novozhilov et al. 2006). It has been collected from Spain (Murcia), Kazakhstan (West Kazakhstan region), and United States (Arizona and New Mexico) (Discover Life 2014).

Didymium squamulosum (Alb. & Schwein.) Fr., Symb. Gasteromyc. 3: 19 (1818).

Specimens examined: **MEXICO, Chihuahua:** Loc. 3, *Larrea tridentata,* 24 January 2009, leg. ML, CS, DS, obtained from moist chamber 25 March 2009 (UES 7585); 20 May 2009, leg. ML, CS & DL, obtained from moist chamber 15 June 2009 (UES 8038, AH 45583); obtained from moist chamber 25 June 2009 (UES 8040); obtained from moist chamber 30 June 2009 (UES 8039, AH 45584); *Larrea tridentata,* 22 October 2009, leg. ML, CS, AS, AG & DL, obtained from moist chamber 27 November 2009 (UES 8440); obtained from moist chamber 9 December 2009 (UES 8441); Loc. 4, *Yucca elata,* 14 February 2009, leg. ML, CS & DS, obtained from moist chamber 18 March 2009 (UES 7586, AH 45582); 20 March 2009 (UES 7813); Loc. 6, *Yucca* sp., 23 March 2009, leg. ML & CS, obtained from moist chamber 20 April 2009 (UES 7686, AH 45581); Loc. 9, *Xanthium* sp. (*Asteraceae*), 22 March 2009, obtained from moist chamber 20 April 2009 (UES 7687).

Observations — *Didymium squamulosum* is a cosmopolitan species (Martin & Alexopoulos 1969). Previously cited for Chihuahua by Lizárraga et al. (2003) and Salazar-Márquez et al. (2014).



Figs 15–24. ***Didymium tehuacanense*** (AH 45559). 15. Sporocarp. 16. Peridium simple and fragile. 17, 18. Columella detail: cylindrical, calcareous, whitish, with ramified and spinose apex. 19. Peridium with starry crystals. 20–22. Capillitium filamentous with abundant nodules (SEM). 23, 24. Spore (SEM). Scale bars. 15–18 = 0.25 mm; 19–22 = 5 µm; 23, 24 = 2 µm.

****Didymium tehuacanense*** Estrada, D. Wrigley & Lado, Fungal Diversity 36: 32 (2009). Figs 15–24

Fructifications stipitate, gregarious, 0.5–1 mm height (Fig. 15). Sporotheca globose to subglobose of 0.3–0.5 mm diam. Peridium simple, iridescent to

grayish, membranous, fragile, persistent on the base of the sporotheca like a collar and covered by starry crystals of whitish calcium carbonate, of bigger size than spores (7–22 µm diam.) Stipe 0.3–0.5 × 0.2–0.4 mm, cylindrical, calcareous, whitish to dark according to the presence of calcium carbonate, wider and darker at the base. Columella 0.2 × 0.1 mm, prominent, calcareous, cylindrical, whitish, with the apex branched and spiny. Hypothallus discoid, membranous. Capillitium formed by thin filaments of 1 µm diam., with thickening or nodules. Spores 8–10 µm diam., dark in mass, violaceous under LM, with a paler area, ornamented with thick warts. Spore ornamentation is formed by irregularly distributed bacula that occasionally join to form short crests as can be seen under SEM.

Specimens examined: **MEXICO, Chihuahua:** Loc. 6, *Agave lecheguilla,* next to *Didymium mexicanum*, 23 March 2009, leg. ML & CS (UES 7577); 25 May 2009, leg. ML, CS & DL (UES 8036); 26 July 2009, leg. ML, CS, DL, DS & AG (UES 8285, AH 45559).

Observations — *Didymium tehuacanense* is characterized by a calcareous stipe and columella, cylindrical columella with a ramified apex and a capillitium with calcareous thickenings.

The above specimens represent a new record for Chihuahua. Previously cited from Oaxaca and Puebla (Estrada-Torres et al. 2009), where it was described as a species new to science.

Echinostelium apitectum K.D. Whitney, Mycologia 72: 954 (1980).

Fructifications stalked, 190–230 µm tall; body spore-like, globose. Stalk 120–210 µm long, filled with granular matter, tapered. Spores 9–11 µm diam., subglobose, uniform wall, hyaline, smooth to weakly warted.

Specimens examined: **MEXICO, Chihuahua:** Loc. 3, *Artemisia filifolia,* 24 January 2009, leg. ML, CS & DS, obtained from moist chamber 16 February 2009 (UES 7822); Loc. 7, *Prosopis glandulosa*, 26 July 2009, leg. ML, CS, DL, DS & AG, obtained from moist chamber 8 August 2009 (UES 8297); *Chilopsis linearis* (Cav.) Sweet (*Bignoniaceae*), 24 May 2009, leg. ML, CS & DL, obtained from moist chamber 29 May 2009 (UES 8050).

Observations — *Echinostelium apitectum* can be confused with fructifications of *E. colliculosum*, which differs by its spores with articulated surfaces and smaller (70–150 µm tall) fructifications (Whitney 1980; Lado & Pando 1997).

Infrequently collected in Mexico, *E. apitectum* is reported for Baja California, Chihuahua, Sonora, and Tlaxcala (Moreno et al. 2007).

Echinostelium arboreum H.W. Keller & T.E. Brooks, Mycologia 68: 1207 (1977)
Figs 13, 14

Peridium persistent, membranous, with a collar; sporotheca 40–50 µm diam.; columella cylindrical ≤25 µm tall, without a spore-like body; capillitium

well developed in our sample, dichotomously branched, not anastomosed. Spores 7–8 µm diam., globose to subglobose, hyaline, smooth to rough and without articulated surfaces.

Specimen examined: **MEXICO, Chihuahua:** Loc. 2, *Artemisia filifolia,* 22 October 2009, leg. ML, CS, AS, AG & DL, obtained from moist chamber 14 November 2009 (UES 8471, AH 45560).

Observations —*Echinostelium arboreum* is known from Baja California, Chihuahua, and Yucatán (Moreno et al. 2007).

Echinostelium colliculosum K.D. Whitney & H.W. Keller, Mycologia 72: 641 (1980).

Specimens examined: **MEXICO, Chihuahua:** Loc. 1, *Artemisia filifolia,* 23 October 2009, leg. ML, CS, AS, AG & DL, obtained from moist chamber 6 November 2009 (UES 8481); cow dung, 14 February 2009, leg. ML, CS & DS, obtained from moist chamber 28 February 2009 (UES 7839); *Prosopis glandulosa*, 14 February 2009, leg. ML, CS & DS, obtained from moist chamber 24 February 2009 (UES 7830); *Artemisia filifolia,* 14 February 2009, leg. ML, CS & DS, obtained from moist chamber 19 February 2009 (UES 7828); Loc. 2, *Artemisia filifolia,* 7 February 2009, leg. ML, CS & DS, obtained from moist chamber 13 February 2009 (UES 7821); *Prosopis glandulosa*, 7 February 2009, leg. ML, CS & DS, obtained from moist chamber 16 February 2009 (UES 7823); *Yucca elata,* 23 July 2009, leg. ML, CS, DL, DS & AG, obtained from moist chamber 3 August 2009 (UES 8288); Loc. 3, *Prosopis glandulosa*, 24 January 2009, leg. ML, CS & DS, obtained from moist chamber 5 February 2009 (UES 7834); *Larrea tridentata*, obtained from moist chamber 6 November 2009 (UES 8477); *Prosopis glandulosa*, obtained from moist chamber 4 February 2009 (UES 7836. *Koeberlinia spinosa,* 20 May 2009, leg. ML, CS & DL, obtained from moist chamber 29 May 2009 (UES 8041); Loc. 4, *Prosopis glandulosa*, 14 February 2009, leg. ML, CS & DS, obtained from moist chamber 24 January 2009 (UES 7824); cow dung, 14 February 2009, leg. ML, CS & DS, obtained from moist chamber 5 March 2009 (UES 7837); *Yucca elata,* obtained from moist chamber 23 February 2009 (UES 7827); *Larrea tridentata,* obtained from moist chamber 26 February 2009 (UES 7838); Loc. 5, *Prosopis glandulosa*, 25 July 2009, leg. ML, CS, DL, DS & AG, obtained from moist chamber 29 May 2009 (UES 8051); *Larrea tridentata,* obtained from moist chamber 8 August 2009 (UES 8293); *Cylindropuntia imbricata*, obtained from moist chamber 10 August 2009 (UES 8218); Loc. 6, *Viguiera stenoloba* S.F.Blake (*Asteraceae*), 23 March 2009, leg. ML & CS, obtained from moist chamber 4 April 2009 (UES 7843); Loc. 7, *Acacia greggii,* 26 July 2009, leg. ML, CS, DL, DS & AG, obtained from moist chamber 3 August 2009 (UES 8296); *Cylindropuntia imbricata*, 22 March 2009, leg. ML & CS, obtained from moist chamber 4 April 2009 (UES 7840); *Porlieria angustifolia,* obtained from moist chamber 4 April 2009 (UES 7841); *Viguiera stenoloba,* obtained from moist chamber 4 April 2009 (UES 7842); 26 October 2009, leg. ML, CS, AS, AG & DL, obtained from moist chamber 6 November 2009 (UES 8484); Loc. 8, *Larrea tridentata*, next to *Dictydiaethalium plumbeum*, 23 March 2009, leg., ML & CS, obtained from moist chamber 4 April 2009 (UES 7576); 24 October 2009, leg. ML, CS, AS, AG & DL, obtained from moist chamber 9 November 2009 (UES 8473); *Porlieria angustifolia,* 23 March 2009, leg. ML & CS, obtained from moist chamber 4 April 2009 (UES 7844); *Viguiera stenoloba* obtained from moist chamber 6 April 2009 (UES 7831); *Yucca* sp., 24 October 2009, leg. ML, CS, AS, AG & DL, obtained from moist chamber 6 November

2009 (UES 8472); obtained from moist chamber 9 November 2009 (UES 8474); Loc. 9, *Prosopis glandulosa*, 25 July 2009, obtained from moist chamber 8 August 2009 (UES 8291); *Acacia greggii,* obtained from moist chamber 10 August 2009 (UES 8231); Loc. 10, *Acacia greggii,* 22 May 2009, leg. ML, CS & DL, obtained from moist chamber 29 May 2009 (UES 8046); *Larrea tridentata*, 25 July 2009, leg. ML, CS, DL, DS & AG, obtained from moist chamber 8 August 2009 (UES 8292).

Observations — *Echinostelium colliculosum* is very difficult to distinguish from *E. coelocephalum* T.E. Brooks & H.W. Keller, since both develop stalked fructifications with a spore-like body on the stipe apex and have globose spores with articulated surfaces. We can differentiate them, because *E. coelocephalum* forms small fructifications 40–70 µm height and very thickened articulated surfaces, while *E. colliculosum* presents larger fructifications of 70–150 µm, and less thickened articulated surfaces (see Whitney 1980).

Echinostelium colliculosum has not been reported as coprophilous by Eliasson & Lundqvist (1979), Eliasson & Keller (1999), or Krug et al. (2004).

Previously cited from Chihuahua by Lizárraga et al. (2003) and Salazar-Márquez et al. (2014).

*****Echinostelium paucifilum*** K.D. Whitney, Mycologia 72: 974 (1980). Figs 25–27

Sporocarps dispersed to gregarious, pinkish brown, stalked, 200–330 µm tall. Stalk 150–210 µm long, filled with granular matter, wider at base with 5–6 µm diam., reducing gradually until the apex to 2–3 µm diam. Peridium fleeting, persisting at base like a collar *ca.* 4 µm diam. Columella cylindrical. Capillitium rudimentary or barely developed. Spores 10–12 µm diam., free, globose to subglobose, hyaline, warted, with one or several protoplasts.

Specimens examined: **MEXICO, Chihuahua:** Loc. 3, *Artemisia filifolia*, 24 January 2009, leg. ML, CS & DS, obtained from moist chamber 27 January 2009, 16 February 2009 (UES 7822); Loc. 8, *Acacia constricta,* 23 May 2009, leg. ML, CS & DL, obtained from moist chamber 29 May 2009 (UES 8044, 8045, AH 45561).

Observations — *Echinostelium paucifilum* is similar to *E. minutum* de Bary, which differs in its larger sporocarps (250–500 µm) and smaller spores (6–8 µm) with articulated surfaces (Whitney 1980).

These collections are new records for Mexico and the Neotropics (Lado & Wrigley de Basanta 2008).

Fuligo cinerea (Schwein.) Morgan, J. Cincinnati Soc. Nat. Hist. 19: 33 (1896). Figs 5, 6

Specimens examined: **MEXICO, Chihuahua:** Loc. 2, cow dung, 22 May 2009, leg. ML, CS & DL, obtained from moist chamber 11 June 2009 (UES 8053, AH 45599); Loc. 4, rabbit dung, 14 February 2009, leg. ML, CS & DS, obtained from moist chamber 27

Figs 25–27. ***Echinostelium paucifilum*** (AH 45561). 25. Sporocarp. 26. Spores. 27. Collar, columella and spore. Scale bars. 25= 50 µm; 26, 27 = 10 µm.

March 2009 (UES 7589, AH 45574); Loc. 7, above ground, 26 October 2009, leg. ML, CS, AS, AG & DL (UES 8486).

Observations — *Fuligo cinerea* generally fruited on herbivore dung following cultivation in moist chamber; macroscopically confused with *Badhamia verrucospora*, *B. spinispora*, and *Physarum apiculosporum* but distinguished from those three species by its spores that are oval to subglobose, warted, and subtly reticulated (Fig. 6). Small fructifications that are convoluted or gyrose on dung could resemble *Physarum gyrosum* Rostaf., but the characteristic *F. cinerea* spores clearly identify it, as noted by Eliasson & Lundqvist (1979) from collections from Tenerife (Canary Islands, Spain) on rabbit dung. We have collected them on rabbit and cow dung.

Previously cited for Chihuahua by Lizárraga et al. (2003) and Salazar-Márquez et al. (2014).

Fuligo septica (L.) F.H. Wigg., Prim. Fl. Holsat.: 112 (1780).

Fructifications showed a whitish aethalium which coincides with *Fuligo septica* var. *candida* (Pers.) R.E. Fr.; spores globose to subglobose with thin warts, 9–11 µm diam., slightly larger than the size (7–9 µm) cited in the literature.

Specimen examined: **MEXICO, Chihuahua:** Loc. 7, above-ground, 26 October 2009, leg. ML, CS, AS, AG & DL (UES 8486, AH 45565).

Observations — This species is widely distributed in Mexico (Moreno et al. 2007).

Licea biforis Morgan, J. Cincinnati Soc. Nat. Hist. 15: 131 (1893).

Sporocarps large, sessile, yellowish brown to dark brown, with a longitudinal centerline dehiscence; spores globose to subglobose, yellow in mass, subtly warted, 10–11 µm diam.

Specimens examined: **MEXICO, Chihuahua:** Loc. 3, *Tamarix chinensis,* 24 January 2009, leg. ML, CS & DS, obtained from moist chamber 5 February 2009 (UES 7596, AH 45597); *Acacia greggii,* obtained from moist chamber 12 August 2009 (UES 8242); *Prosopis glandulosa*, 24 January 2009, leg. ML, CS & DS, obtained from moist chamber 26 February 2009 (UES 7602); Loc. 6, *Viguiera stenoloba,* 23 March 2009, obtained from moist chamber 20 April 2009 (UES 7610); Loc. 7, *Porlieria angustifolia,* 22 March 2009, obtained from moist chamber 13 April 2009 (UES 7606); *Acacia greggii,* obtained from moist chamber 8 August 2009 (UES 8300); Loc. 10, *Cylindropuntia* sp., 21 March 2009, leg. ML & CS, obtained from moist chamber 8 April 2009 (UES 7603, 7604); *Larrea tridentata,* obtained from moist chamber 8 April 2009 (UES 7605).

Observations — *Licea biforis* has been cultivated in moist chambers over different tree barks and bushes, including succulent desert plants such as members of the *Cactaceae* and *Liliaceae*.

Two species have been described with a succulenticolous habit similar to *Licea biforis: Licea succulenticola* (Mosquera et al. 2003) and *Licea capacia* Oltra & G. Moreno (Moreno & Oltra 2014).

Licea biforis has been previously cited for Chihuahua and Sonora (Moreno et al. 2007).

Licea denudescens H.W. Keller & T.E. Brooks, Mycologia 69: 668 (1977).

Specimens examined: **MEXICO, Chihuahua:** Loc. 2, 7 February 2009, leg. ML, CS & DS, obtained from moist chamber 13 March 2009 (UES 7614); obtained from moist chamber 23 February 2009 (UES 7717); 22 October 2009, leg. ML, CS, AS, AG & DL, obtained from moist chamber 9 December 2009 (UES 8338); Loc. 3, *Prosopis glandulosa*, 24 January 2009, leg. ML, CS & DS, obtained from moist chamber 16 February 2009 (UES 7612); obtained from moist chamber 23 February 2009 (UES 7718); obtained from moist chamber 28 February 2009 (UES 7613, AH 45596); 22 October 2009, leg. ML, CS,

AS, AG & DL, obtained from moist chamber 21 November 2009 (UES 8488); obtained from moist chamber 27 November 2009 (UES 8502); obtained from moist chamber 9 December 2009 (UES 8503); Loc. 7, *Acacia greggii,* 26 July 2009, leg. ML, CS, DL, DS & AG, obtained from moist chamber 29 August 2009 (UES 8340); 26 October 2009, leg. ML, CS, AS, AG & DL, obtained from moist chamber 9 December 2009 (UES 8500); *Chilopsis linearis,* 26 July 2009, leg. ML, CS, DL, DS & AG, obtained from moist chamber 29 August 2009 (UES 8341); *Fouquieria splendens,* 26 October 2009, leg. ML, CS, AS, AG & DL, obtained from moist chamber 27 November 2009 (UES 8498); *Cylindropuntia* sp., obtained from moist chamber 9 December 2009 (UES 8501).

Observations — Recently described for Chihuahua by Salazar-Márquez et al. (2014), and previously from Puebla (Estrada-Torres et al. 2009) and Sonora (Esqueda et al. 2013).

Licea kleistobolus G.W. Martin, Mycologia 34: 702 (1942).

Specimens examined: **MEXICO, Chihuahua:** Loc. 1, 14 February 2009, leg. ML, CS & DS, obtained from moist chamber 25 February 2009 (UES 7617); obtained from moist chamber 26 February 2009 (UES 7619); obtained from moist chamber 27 February 2009 (UES 7618); obtained from moist chamber 2 March 2009 (UES 7621, AH 45595); obtained from moist chamber 12 March 2009 (UES 7620); 23 July 2009, leg. ML, CS, DL, DS & AG, obtained from moist chamber 5 August 2009 (UES 8302); obtained from moist chamber 8 August 2009 (UES 8303); *Prosopis glandulosa*, obtained from moist chamber 8 August 2009 (UES 8304); Loc. 2, *Artemisia filifolia,* 7 February 2009, leg. ML, CS & DS, obtained from moist chamber 12 February 2009 (UES 7616); obtained from moist chamber 16 February 2009 (UES 7714); Loc. 3, *Prosopis glandulosa*, 24 January 2009, leg. ML, CS & DS, obtained from moist chamber 22 February 2009 (UES 7615); 23 July 2009, leg. ML, CS & DL, DS & AG, obtained from moist chamber 8 August 2009 (UES 8301); Ibidem, *Tamarix chinensis,* 25 October 2009, leg. ML, CS, AS, AG & DL, obtained from moist chamber 21 November 2009 (UES 8495); Loc. 6, *Viguiera stenoloba,* 25 May 2009, leg. ML, CS & DL, obtained from moist chamber 29 May 2009 (UES 8058); *Yucca* sp., 26 July 2009, obtained from moist chamber 22 August 2009 (UES 8345); Loc. 9, *Acacia greggii*, 25.X.20009, leg. ML, CS, AS, AG & DL, obtained from moist chamber 14 November 2009 (UES 8493); obtained from moist chamber 21 November 2009 (UES 8494); Loc. 10, *Larrea tridentata*, 25 October 2009, obtained from moist chamber 21 November 2009 (UES 8496).

Observations — Previously cited from Chihuahua by Lizárraga et al. (2003) and Salazar-Márquez et al. (2014).

Licea succulenticola Mosquera, Lado, Estrada & Beltrán-Tej., Anales Jard. Bot. Madrid 60: 4 (2003).

Specimens examined: **MEXICO, Chihuahua:** Loc. 6, *Agave lecheguilla,* 23 March 2009, leg. ML & CS (UES 7622, AH 45594; UES 7638 next to *Perichaena vermicularis*).

Observations — Recently described from Chihuahua by Salazar-Márquez et al. (2014). It might be widely distributed in Mexico but has been unobserved because of its small size.

**Lycogala exiguum* Morgan, J. Cincinnati Soc. Nat. Hist. 15: 134 (1893).

Aethalium small (2–4 mm diam.); peridium ornamented by outgoing reddish brown warts which form a compartmented structure that gives a false pluricellular appearance under LM; spores 5–6 µm diam.; capillitium abundant, 2–10 µm diam., filamentous, tubular, with sinuous walls.

Specimen examined: **MEXICO, Chihuahua:** Loc. 6, *Yucca* sp., 23 March 2009, leg. ML & CS (UES 7623, AH 45593).

Observations — See Hooff (2014) for diagnostic differences among species of *Lycogala* Adans.

Lycogala exiguum has been reported for Jalisco, Nuevo León, Quintana Roo, Tabasco, Veracruz, and Yucatán (Moreno et al. 2007).

Perichaena pachyderma D.W. Mitch., G. Moreno & Lizárraga, Bol. Soc. Micol. Madrid 35: 104 (2011). Figs 28–33

Fructifications sessile, globose to subglobose, solitary to gregarious. Sporotheca 0.1–0.3 mm diam., dark yellow in youth, to dark brown when maturing. Peridium simple, 3–4.5 µm thick, externally covered by granular matter; the internal surface presents small warts which binding with a reticulated appearance (not noted by Mitchell et al. (2011) in his type description). Capillitium not observed. Spores 10–11 µm diam., orange-yellow in mass, light yellow under LM, globose, subtly spinulose. Spore ornamentation is formed by irregular distributed bacula.

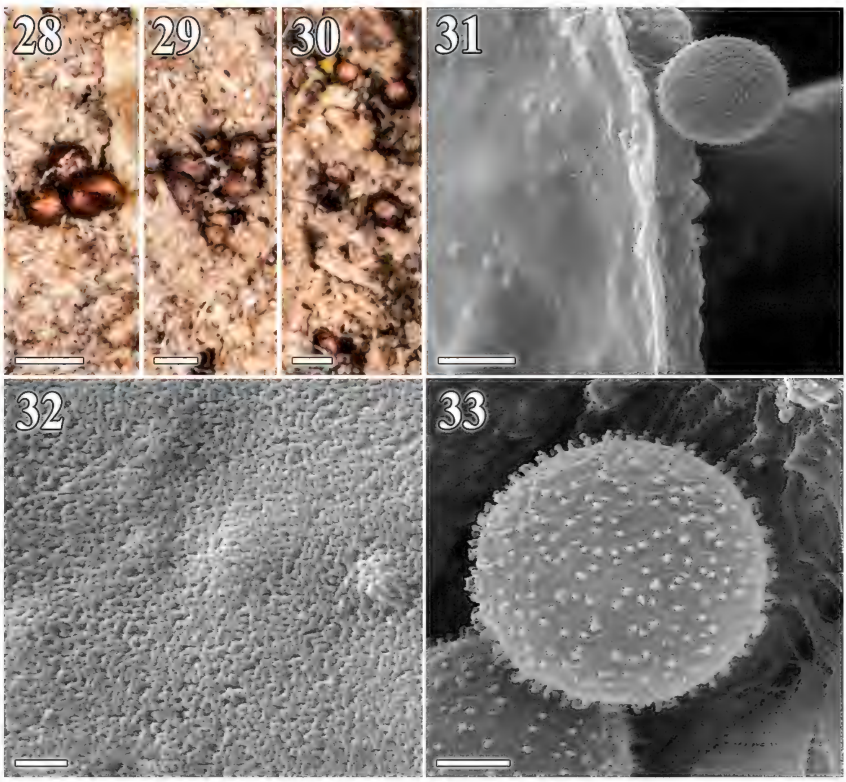
Specimens examined: **MEXICO, Chihuahua:** Loc. 3, cow dung, 24 January 2009, leg. ML, CS & DS, obtained from moist chamber 13 February 2009 (UES 7591); obtained from moist chamber 17 February 2009 (UES 7592); Loc. 5, 23 March 2009, leg. ML & CS, obtained from moist chamber 6 April 2009 (UES 7594, AH 45562); Loc. 6, 23 March 2009, obtained from moist chamber 6 April 2009 (UES 7595); Loc. 9, 24 May 2009, leg. ML, CS & DL, obtained from moist chamber 29 May 2009 (UES 8057).

Observations — Fructifications cultivated in moist chamber over herbivore dung were similar to *Licea tenera* E. Jahn (studied by Martin & Alexopoulos, 1969), but *L. tenera* has smooth spores and our collections have spinulose or verrucose ornamentation. The specimens also resemble *Perichaena* cf. *liceoides* Rostaf. as described by Eliasson & Lundqvist (1979) but which has a poorly developed capillitium, absent in our collections.

Although it is very possible that this coprophilous species has a wider distribution, it is only known from the United States (New Mexico) and Mexico (Baja California and Chihuahua) (Mitchell et al. 2011).

Perichaena quadrata T. Macbr., N. Amer. Slime-Moulds: 184 (1899). Figs 34–42

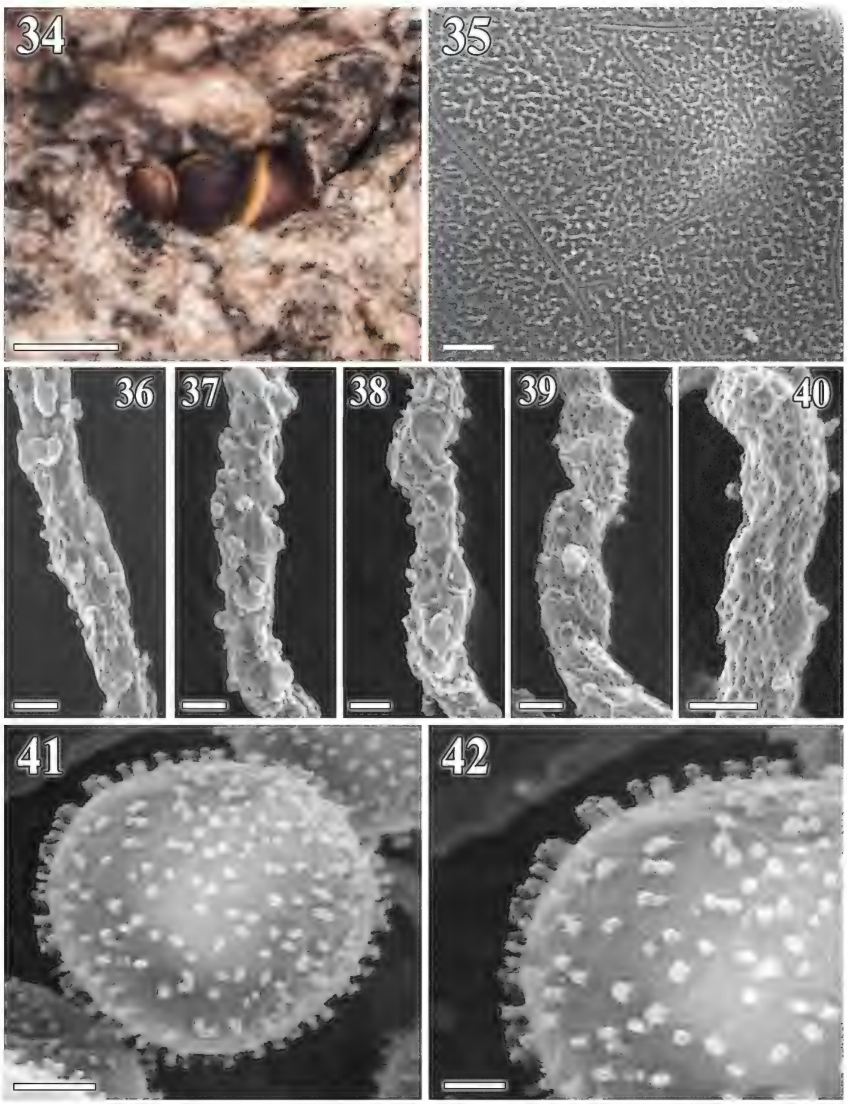
Fructifications gregarious (rarely solitary), rounded to pulvinate, convex, 0.3–0.5 cm diam., reddish brown to more or less blackish when maturing,



Figs 28–33. ***Perichaena pachyderma*** (AH 45562). 28–30. Sporocarps gregarious to solitary. 31. Peridium thick (SEM). 32. Peridium internal surface detail (SEM). 33. Spore (SEM). Scale bars: 28–31 = 0.1 mm; 31 = 5 µm; 32 = 1 µm; 33 = 2 µm.

dehiscence circumscissile and separating the peridium like a convex operculum. Peridium internal surface warted, with the warts joining to form sometimes anastomosing sinuous structures. Capillitium abundant, filiform, 1.5–2 µm diam.; under SEM, the ornamentation seen as thick isolated more or less abundant warts and a reticle full of variably wide meshes. Spores 9–11 µm diam., yellowish, spinulose (LM); under SEM, ornamentation is formed by uniformly distributed bacula and a weakly coralloid apex.

Specimens examined: **MEXICO, Chihuahua:** Loc. 2, *Yucca elata,* 7 February 2009, leg. ML, CS & DS (UES 7626); cow dung, 22 May 2009, leg. ML, CS & DL, obtained from moist chamber 30 June 2009 (UES 8066); Loc. 4, *Tamarix chinensis,* 14 February 2009, leg. ML, CS & DS, obtained from moist chamber 25 March 2009 (UES 7627, AH 45563); Loc. 6, *Agave lecheguilla,* 26 October 2009, leg. ML, CS, AS, AG & DL (UES 8511, AH 45564); Loc. 8, *Yucca* sp., 25 July 2009, leg. ML, CS, AG, DL & DS, obtained from moist chamber 22 August 2009 (UES 8354).



Figs 34–42. ***Perichaena quadrata*** (AH 45563). 34. Sporocarps gregarious. 35. Peridium, internal surface detail (SEM). 36–40. Variable capillitium reticulate ornamentation (SEM). 41, 42. Spore ornamentation (SEM, detail). Scale bars: 34 = 1 mm; 35–40, 42 = 1 μm; 41 = 2 μm.

Observations — *Perichaena quadrata* has been confused or synonymized with *P. depressa* Lib., which presents larger sporocarps (0.5–1.2 cm diam.), also with a circumscissile peridium but flat apex; capillitium 2–2.5(–3) μm diam.,

filiform, and without a reticulated ornamentation, as observed by Keller & Eliasson (1992), whose concept we follow.

Rammeloo (1981) examined under the SEM two African species close to *P. quadrata* that fructify over different substrata: *P. areolata* Rammeloo (leaves), and *P. dictyonema* Rammeloo (crusts and wood debris); *P. dictyonema* is morphologically very similar to *P. quadrata,* and they might represent the same species.

Perichaena quadrata has been recorded from Puebla (Estrada-Torres et al. 2009) and Chihuahua (Lizárraga et al., 2015). It was cited as coprophilous by Eliasson & Lundqvist (1979), Eliasson & Keller (1999), and Krug et al. (2004). Previously cited from Chihuahua by Lizárraga et al. (2015).

*****Physarum apiculosporum*** Härk., Karstenia 18: 24 (1978). Figs 7, 8

Plasmodiocarps gregarious, sessile, pulvinate, sinuous, curved or reticulated, 0.3–0.6 mm wide × 4.5 mm long. Peridium greyish to white, double, with the two layers closely associated, outer layer with abundant lime; inner layer thin, membranous. Dehiscence irregular. Capillitium formed by scarce thin filaments, white, rounded to angular lime nodes. Spores black in mass, reddish brown, 10–14 × 8–10 µm, ellipsoid to oval, smooth with an apiculus and a longitudinal ridge by LM; spore surface smooth under SEM.

Specimen examined: **MEXICO, Chihuahua:** Loc. 7, cow dung, 26 October 2009, leg. ML, CS, AS, AG & DL, obtained from moist chamber 9 December 2009 (UES 8530, AH 45566).

Observations — *Physarum apiculosporum* is characterized by forming plasmodiocarps and spores oval to ellipsoid, smooth, with two pointed ends, one at each ending (Härkönen 1978, Moreno et al. 2011). This species may be confused with *Badhamia verrucospora;* both share habit and spore ornamentation, but *B. verrucospora* differs in warted spores that are strongly marked over half of the surface, while the remainder is smooth, even under SEM (Moreno et al. 2011).

Physarum apiculosporum is a new record for Mexico and the Neotropics (Lado & Wrigley de Basanta 2008).

Physarum decipiens M.A. Curtis, Amer. J. Sci. Arts, Ser. 2, 6: 352 (1848).

Specimens examined: **MEXICO, Chihuahua:** Loc. 1, *Prosopis glandulosa*, 14 February 2009, leg. ML, CS & DS, obtained from moist chamber 21 February 2009 (UES 7661); obtained from moist chamber 28 February 2009 (UES 7662); obtained from moist chamber 6 March 2009 (UES 7663); Loc. 2, 7 February 2009, leg. ML, CS & DS, obtained from moist chamber 14 March 2009 (UES 7659); Loc. 3, *Prosopis glandulosa*, 24 January 2009, leg. ML, CS & DS, obtained from moist chamber 2 March 2009 (UES 7658); Loc.

4, *Larrea tridentata,* 14 February 2009, 16 February 2009, leg. ML, CS & DS, obtained from moist chamber 18 March 2009 (UES 7660); *Fouquieria splendens,* 26 July 2009, leg. ML, CS, DL, DS & AG, obtained from moist chamber 10 August 2009 (UES 8236); Loc. 7, *Viguiera stenoloba,* 22 March 2009, leg. ML & CS, obtained from moist chamber 20 April 2009 (UES 7664); *Acacia greggii,* 24 May 2009, leg. ML, CS & DL, obtained from moist chamber 11 June 2009 (UES 8074); *Acacia greggii*, 26 July 2009, leg. ML, CS, DL, AG & DS, obtained from moist chamber 8 August 2009 (UES 8215); obtained from moist chamber 10 August 2009 (UES 8235); 26 October 2009, leg. ML, CS, AS, AG & DL, obtained from moist chamber 6 November 2009 (UES 8515); obtained from moist chamber 27 November 2009 (UES 8529, AH 45592); Loc. 10, *Prosopis glandulosa*, 26 July 2009, leg. ML, CS, DL, DS & AG, obtained from moist chamber 8 September 2009 (UES 8366, AH 45591).

Observations — *Physarum decipiens* is morphologically highly variable. Previously reported from Chihuahua (Lizárraga et al. 2003), Oaxaca, Puebla (Estrada-Torres et al. 2009), Sonora (Esqueda et al. 2013), and Tlaxcala (Rodríguez-Palma et al. 2002).

Physarum leucophaeum Fr., Symb. Gasteromyc. 3: 24 (1818).

Specimens examined: **MEXICO, Chihuahua:** Loc. 1, cow dung, 14 February 2009, leg. ML, CS & DS, obtained from moist chamber 14 March 2009 (UES 7665); rabbit dung, obtained from moist chamber 2 April 2009 (UES 7653, AH 45567); 22 May 2009, leg. ML, CS & DL, obtained from moist chamber 22 June 2009 (UES 8092); Loc. 3, *Prosopis glandulosa*, 20 May 2009, leg. ML, CS & DL, obtained from moist chamber 25 June 2009 (UES 8091, AH 45568); Loc. 6, *Prosopis glandulosa*, 25 June 2009, obtained from moist chamber 3 June 2009 (UES 8080, AH 45590); Loc. 8, *Cylindropuntia imbricata*, 25 July 2009, leg. ML, CS, DL, DS & AG, obtained from moist chamber 5 August 2009 (UES 8211).

Observations — *Physarum leucophaeum* is very close to *P. robustum* and *P. notabile* T. Macbr., two species that appear confused in the literature. We follow Nannenga-Bremekamp (1991) for distinguishing *P. leucophaeum* from *P. robustum: P. leucophaeum* presents a capillitium with very few calcium carbonate nodules, which are small and round, while *P. robustum* has a capillitium with bigger and abundant fusiform calcium carbonate nodules that generally join at sporotheca center to form a whitish pseudocolumella (see photographs in Poulain et al. 2011). Our specimens were collected on cow and rabbit dung. This coprophilous habit was not indicated in Eliasson & Lundqvist (1979), Eliasson & Keller (1999), and Krug et al. (2004). Previously cited from Chihuahua by Lizárraga et al. (2003).

Physarum pusillum (Berk. & M.A. Curtis) G. Lister, Monogr. Mycetozoa, 2nd Ed.: 64 (1911).

Specimens examined: **MEXICO, Chihuahua:** Loc. 1, cow dung, obtained from moist chamber 30 June 2009 (UES 8086); Loc. 2, *Yucca elata,* 7 February 2009, leg. ML, CS

& DS, obtained from moist chamber 24 March 2009 (UES 7670); obtained from moist chamber 24 March 2009 (UES 7669); 23 July 2009, leg. ML, CS, DL, DS & AG, obtained from moist chamber 15 August 2009 (UES 8360); Loc. 3, *Tamarix chinensis,* 24 January 2009, leg. ML, CS & DL, obtained from moist chamber 25 February 2009 (UES 7650); obtained from moist chamber 2 March 2009 (UES 7651); *Larrea tridentata*, obtained from moist chamber 3 March 2009 (UES 7646); *Koeberlinia spinosa,* 14 February 2009, leg. ML, CS & DS, obtained from moist chamber 30 June 2009 (UES 8081); Loc. 4, *Larrea tridentata,* leg. ML, CS & DS, obtained from moist chamber 18 March 2009 (UES 7652); Loc. 5, *Cylindropuntia imbricata*, 22 May 2009, obtained from moist chamber 11 June 2009 (UES 8073); Loc. 7, *Chilopsis linearis,* 24 May 2009, leg. ML, CS & DL, obtained from moist chamber 15 June 2009 (UES 8094); Loc. 8, *Opuntia* sp., 24 October 2009, leg. ML, CS, AS, AG & DL (UES 8378); inflorescence of *Yucca* sp., 23 May 2009, obtained from moist chamber 29 May 2009 (UES 8071); obtained from moist chamber 3 June 2009 (UES 8096, AH 45587); obtained from moist chamber 22 June 2009 (UES 8093); Loc. 10, *Koeberlinia spinosa,* 25 July 2009, obtained from moist chamber 22 August 2009 UES 8363); *Acacia constricta*, obtained from moist chamber 8 September 2009 (UES 8364).

Observations — *Physarum pusillum* is frequently observed in moist chamber cultures. Previously reported for Chihuahua by Lizárraga et al. (2003).

Physarum robustum (Lister) Nann.-Bremek., Proc. Kon. Ned. Akad. Wetensch., C 76: 484 (1973).

Specimens examined: **MEXICO, Chihuahua:** Loc. 4, *Tamarix chinensis,* 22 May 2009, leg. ML, CS & DL, obtained from moist chamber 30 June 2009 (UES 8084, AH 45569); Loc. 6, *Prosopis glandulosa*, 26 July 2009, leg. ML, CS, DL, DS & AG, obtained from moist chamber 10 August 2009 (UES 8234, AH 45570).

Observations — For *Physarum robustum* we follow the concept of Nannenga-Bremekamp (1991). Differences with closely related species like *P. album* (Bull.) Chevall., *P. leucophaeum*, and *P. leucopus* Link have been described for Sonora (Moreno et al. 2004) and Chihuahua (Lizárraga et al. 2015).

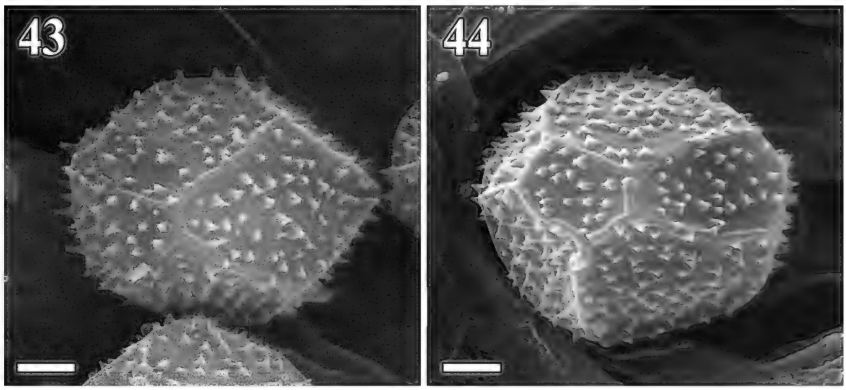
****Physarum spectabile*** Nann.-Bremek., Lado & G. Moreno, Proc. Kon. Ned. Akad. Wetensch., C 87: 91 (1984).

Specimens examined: **MEXICO, Chihuahua:** Loc. 6, *Agave lecheguilla,* 23 March 2009 (UES 7668); *Yucca* sp., 23 March 2009 (UES 7667, AH 45571).

Observations — *Physarum spectabile* is a common succulenticolous species. Recorded for Oaxaca and Puebla by Estrada-Torres et al. (2009)

Physarum straminipes Lister, J. Bot. 36: 163 (1898). Figs 43, 44

Fructifications whitish, stipitate with a false stalk, which is an extension of a whitish to yellowish hypothallus; capillitium with whitish nodules, globose, more abundant at the center of the sporocarp; spores polygonal, dark, with scarce and thick spines of irregular distribution (see SEM).



Figs 43, 44. ***Physarum straminipes*** (AH 45572). Spores (SEM). Scale bars = 2 μm.

Specimen examined: **MEXICO, Chihuahua:** Loc. 9, rabbit dung, 22 March 2009, leg. ML & CS, obtained from moist chamber 1 May 2009 (UES 7671, AH 45572).

Observations — *Physarum straminipes* was not noted as coprophilous by Eliasson & Lundqvist (1979), Eliasson & Keller (1999), or Krug et al. (2004). Previously reported for Baja California, Sonora (Moreno et al. 2007), and Querétaro (Tapia et al. 2008).

Protophysarum phloiogenum M. Blackw. & Alexop., Mycologia 67: 33 (1975).

Specimens examined: **MEXICO, Chihuahua:** Loc. 1, *Atriplex canescens* (Pursh) Nutt. (*Amaranthaceae*), obtained from moist chamber 6 March 2009 (UES 7684); 24 July 2009, leg. ML, CS, DL, DS & AG, obtained from moist chamber 12 August 2009 (UES 8238); Loc. 3, *Tamarix chinensis,* 24 January 2009, leg. ML, CS & DS, obtained from moist chamber 3 February 2009 (UES 7673); obtained from moist chamber 4 February 2009 (UES 7674); obtained from moist chamber 5 February 2009 (UES 7675); obtained from moist chamber 11 February 2009 (UES 7676); *Atriplex canescens,* 22 October 2009, leg. ML, CS, AS, AG & DL, obtained from moist chamber 9 November 2009 (UES 8532); obtained from moist chamber 14 November 2009 (UES 8533); obtained from moist chamber 21 November 2009 (UES 8522); Loc. 4, *Atriplex canescens,* 14.II.2209, leg. ML, CS & DS, obtained from moist chamber 23 February 2009 (UES 7677); obtained from moist chamber 2 March 2009 (UES 7678); obtained from moist chamber 11 March 2009 (UES 7679); 22 May 2009, leg. ML, CS & DL, obtained from moist chamber 29 May 2009 (UES 8097); obtained from moist chamber 1 June 2009 (UES 8098); obtained from moist chamber 3 June 2009 (UES 8099); obtained from moist chamber 11 June 2009 (UES 8100); obtained from moist chamber 15 June 2009 (UES 8101); decomposing wood, obtained from moist chamber 3 June 2009 (UES 8102); *Yucca elata,* 14.II.2209, leg. ML, CS & DS, obtained from moist chamber 27 February 2009 (UES 7680); obtained from moist chamber 2 March 2009 (UES 7681); rabbit dung, obtained from moist chamber 20 March 2009 (UES 7682).

Observations — *Protophysarum phloiogenum* appears frequently on such substrates as *Atriplex canescens, Tamarix chinensis, Yucca elata*, and even on rabbit dung when cultured in moist chambers. A complete study of this species, including its variable spore ornamentation, was carried out by Castillo et al. (1998). Previously cited for Baja California, Chihuahua (Moreno et al. 2007), and Sonora (Esqueda et al. 2013).

Trichia agaves (G. Moreno, Lizárraga & Illana) Mosquera, Lado, Estrada & Beltrán-Tej., Cuad. Trab. Fl. Micol. Ibér. 16: 82 (2001).

Specimen examined: **MEXICO, Chihuahua:** Loc. 6, *Agave lecheguilla,* leg. ML, CS, DL, DS & AG, 26 July 2009 (UES 8214, AH 45573).

Observations — This species was described by Moreno et al. (2000) from several collections from Mexico and Spain on members of the *Cactaceae* and *Liliaceae*. It is frequently collected in desert areas and was recently reported for Cuba (Camino et al. 2008).

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Three *Trichoderma* species associated with wood discoloration in South Korea

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Abstract — Three *Trichoderma* species previously recorded as new records from South Korea, *T. dorotheae, T. gamsii,* and *T. koningiopsis*, are described and illustrated.

Key words — ascomycetes, *Hypocrea*, phylogeny, taxonomy

Introduction

Trichoderma Pers. [= *Hypocrea* Fr.] is a cosmopolitan genus of fungi (*Hypocreaceae, Ascomycota*) comprising more than one hundred species (Druzhinina et al. 2006), which can be isolated from soil, wood, and various other substances, even including other fungi (Druzhinina et al. 2011).

In South Korea, about 20 *Trichoderma* species have been reported (Lee et al. 2003; Park et al. 2005, 2006; Cho et al. 2007; Huh et al. 2011). Ten *Trichoderma* species isolated from wood were identified by Huh et al. (2011), who examined the culture characteristics and analyzed the ITS and TEF sequences of the isolates; they reported three species—*Trichoderma dorotheae, T. gamsii*, and *T. koningiopsis*—as new records from South Korea but provided no microscopic descriptions. Here we confirm the identifications of these *Trichoderma* records through macro- and microscopical observations and an expanded phylogenetic analysis using translation elongation factor-1α (TEF) gene region sequences.

Materials & methods

Analysis of phenotype

Six isolates of the three *Trichoderma* species were obtained from the Korea University Culture Collection, Seoul, Republic of Korea (KUC). Cultures used for

micromorphology were grown on corn meal dextrose agar (CMD: cornmeal 20 g, glucose 20 g, agar 18 g, distilled water 1000 ml), potato dextrose agar (PDA: Difco™ potato dextrose agar 39 g, distilled water 1000 ml) or Synthetic Nitrogenpoor or Nutrient poor agar (SNA: sucrose 0.2 g, glucose 0.2 g, KNO_3 1 g, KH_2PO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, NaCl 0.5 g, agar 12 g, distilled water 1000 ml). The fungi were cultured at 25 or 30°C for 7d because their optimal growth temperatures were placed at 25–30°C (Huh et al. 2011). Microscopic characters were observed with an Olympus BX51 light microscope. Pictures of conidiophores and conidia were taken using the same microscope mounted with an Olympus DP20 microscopic camera. Measurements were made from 3% KOH mounts. At least 30 units of each parameter were measured for each collection. To make reliable data, 5% of the measurements from each end of the range were removed. The isolates were deposited at the National Institute of Biological Resources, Incheon, South Korea (NIBR).

Phylogenetic analysis

The TEF region was used for phylogenetic analysis of the *Trichoderma* species (Samuels et al. 2006). For three isolates (KUC1747, KUC5027, and KUC5063), TEF sequences were obtained from Huh et al. (2011). For the other three isolates (KUC3025, KUC5028, and KUC5064), DNA samples were extracted and the TEF region was amplified according to Huh et al. (2011). The TEF sequences were proofread and aligned with selected GenBank reference sequences using MAFFT 7.130 (Katoh & Standley 2013) and modified manually with MacClade 4.08 (Maddison & Maddison 2005). Datasets were tested by MrModeltest 2.3 using the AIC criteria with default options (Nylander 2004). The HKY + G model was chosen under the AIC criteria as a result of the test. Bayesian analysis was performed with MrBayes 3.2.1 (Ronquist et al. 2012). A phylogenetic tree was created according to Jang et al. (2012).

Taxonomy

Trichoderma dorotheae Samuels & Dodd, Stud. Mycol. 56: 112 (2006) Fig. 1a–c,j,k

Conidiophores lacking a discernible main axis and branches not obviously paired or unilaterally branched, re-branching to form second branches, which terminate in slightly divergent whorls of 3–4 phialides; conidiophores often arising from pustules, 2.0–2.5 µm wide. Phialides lageniform, swollen in the middle, 5.5–10.5 µm long, 2.5–3.5 µm at the widest point. Conidia broadly ellipsoidal, smooth, 3.2–4.0 × 2.2–3.5 µm.

Culture characteristics — Optimum temperature for growth on PDA and SNA 25°C. Colonies grown on SNA at 25°C remaining sterile. A white mycelium covering the 9 cm diam Petri plate at 25°C after 1 wk in darkness; No pigment diffusing through the agar; no distinctive odor. Colonies grown on PDA at 25°C in darkness forming conidia only after 96 h. A dense white mycelium covering the 9 cm diam Petri plate at 25°C after 1 wk in darkness; conidia moderate, bluish green. No pigment diffusing through the agar; no

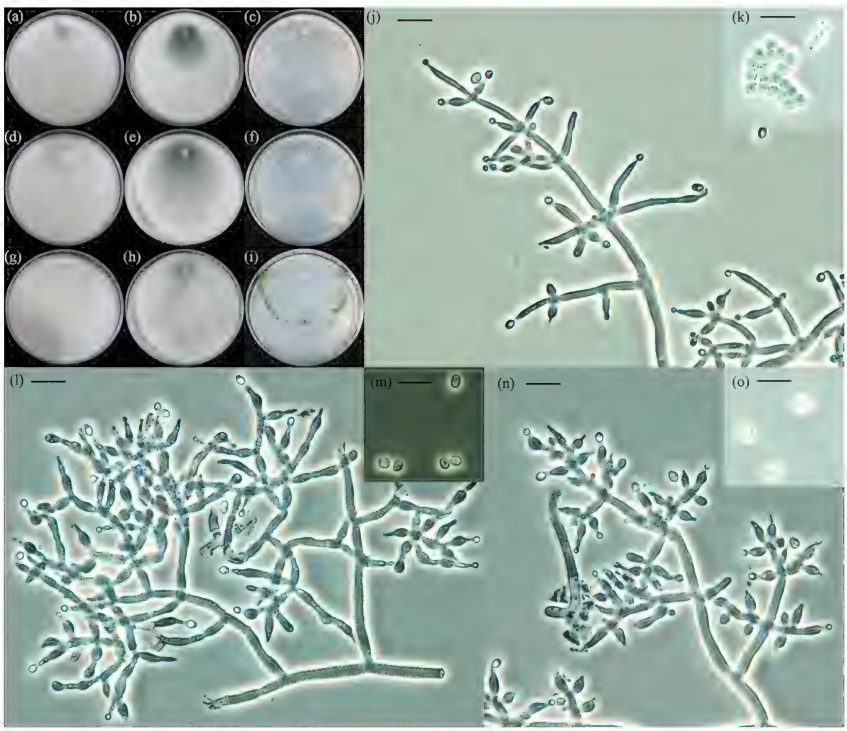

FIG. 1. ***Trichoderma dorotheae***: growth on (a) PDA; (b) CMD; (c) SNA. ***Trichoderma gamsii***: growth on (d) PDA; (e) CMD; (f) SNA. ***Trichoderma koningiopsis***: growth on (g) PDA; (h) CMD; (i) SNA. [All cultures in 9-cm-diam Petri dishes after 96 h at 25°C]. ***Trichoderma dorotheae***: (j) conidiophores; (k) conidia. ***Trichoderma gamsii***: (l) conidiophores; (m) conidia. ***Trichoderma koningiopsis***: (n) conidiophores; (o) conidia. Scale bar = 10 μm.

distinctive odor. Colonies grown on CMD at 25°C in darkness filling the Petri plate within 1 wk, colonies remaining sterile.

DISTRIBUTION — New Zealand, Australia (Samuels et al. 2006), and South Korea.

SPECIMEN EXAMINED: **KOREA, JEOLLABUK-DO,** Iksan-si, a log of *Pinus radiata*, August 2008, Jae-Jin Kim (NIBR-FG0000120470, culture KUC5027, GenBank HM534661).

REMARKS — The TEF sequences of *T. dorotheae* KUC5027 (503 bp) and other *T. dorotheae* strains were monophyletic with high posterior probability support (PP = 1). *Trichoderma dorotheae* can cause light green discoloration, but wood-discoloration rate was low (Huh et al. 2011). The Korean isolate of *T. dorotheae* has a relatively high antagonistic ability against other wood-damaging fungi, so it has potential as a biological control agent (Lee et al. 2012).

Trichoderma gamsii Samuels & Druzhin., Stud. Mycol. 56: 168 (2006) Fig. 1d–f,l,m

Conidiophores more or less uniformly branched for a short distance, branches often arising from swollen nodes on the conidiophore, a single phialide forming at the tip, 2.0–3.0 µm wide. Phialides lageniform, at most slightly swollen in the middle, 7.0–10.5 µm long, 2.5–3.0 µm at the widest point, 1.5–2.5 µm at the base; Intercalary phialides common. Conidia ellipsoidal, smooth, 3.3–3.9 × 2.0–2.8 µm.

Culture characteristics Optimum temperature for growth on PDA and SNA 25–30°C; after 72 h on PDA colony radius reaching 50–52 mm, on SNA colony radius 39-42 mm. On PDA at 25°C and 30°C after 1 wk in darkness typically a dense white mycelium covering the 9 cm diam Petri plate; colonies remaining sterile. More or less strong coconut-like odor. On SNA at 25°C and 30°C covering the 9 cm diam Petri plate after 1 wk in darkness; conidia forming abundantly, grayish green, in broad sheets and sometimes confluent pustules sporadically produced, 1–2 mm diam. No pigment diffusing through the agar, no distinctive odor.

Distribution — Australia, Central Russia, Italy, U.S.A. (Jaklitsch et al. 2006), and South Korea.

Specimen examined: **KOREA, Jeollabuk-do**, Iksan-si, a log of *Pinus radiata*, August 2008, Jae-Jin Kim (NIBR-FG0000120471, culture KUC1747, GenBank HM534663).

Remarks — The most conspicuous characters of *T. gamsii* were its coconut-like odor and abundant aerial mycelia on CMD or PDA. As observation of conidiophores of *T. gamsii* on CMD or PDA was obscured by their aerial mycelia, most measurements were determined on SNA.

The macro- and micromorphological features of the Korean isolate agreed well with the protologue description, except for the protologue conidia being longer ((3.2–)4.0–5.0(–6.2) µm; Jaklitsch et al. 2006). The TEF sequences of *T. gamsii* KUC1747 (594 bp) and other *T. gamsii* strains were monophyletic with high support (PP = 1).

Trichoderma gamsii can cause green discoloration on wood surfaces with high wood-discoloration rate (Huh et al. 2011). On the other hand, *T. gamsii* has strong antagonistic properties against wood-rotting fungi and sap-staining fungi (Lee et al. 2012). *Trichoderma gamsii* KUC1747 produces 6-pentyl-α-pyrone that is a known antagonistic metabolite against sap-staining fungi (Hong et al. 2014).

Trichoderma koningiopsis Samuels, C. Suárez & H.C. Evans, Stud. Mycol. 56: 117 (2006) Fig. 1g–i,n,o

Conidiophores 2.5–3.5 µm wide, branches formed from the interior of pustules, the first branches rebranching or making phialides directly, the

second branches tending to be paired; all fertile branches terminating in a whorl of phialides. Phialides lageniform, held in whorls of 2–5, sometimes several phialides arising from the same point and crowded, 5.5–7.5 µm long, 2.5–3.0 µm at the widest point. Conidia broadly ellipsoidal, smooth, 2.8–3.6 × 2.6–3.0 µm.

Culture characteristics Optimum temperature for growth on PDA and SNA 25–30°C. Colonies grown on PDA in darkness sometimes forming conidia after 48 h at 25 and 30°C in a concentric ring. A dense white mycelium covering the 9 cm diam Petri plate at 25°C and 30°C after 1 wk in darkness; conidia moderate, sometimes abundant at 30°C from some isolates. No pigment diffusing through the agar; rarely a faint coconut odor detected. Colonies grown on SNA in darkness for 96 h producing abundant aerial mycelium; conidia tending to be uniformly dispersed in the aerial mycelium in 1–2 broad concentric rings, sometimes forming cottony pustules. Conidial masses dark green. No pigment diffusing through the agar; no distinctive odor. Colonies grown on CMD in darkness a dense white mycelium covering the 9-cm-diam Petri plate within 1 wk at 25 °C. Conidial production restricted near the inoculated spot of the colony with a tendency to form highly compact to cottony, 1–2 mm diam pustules; Often long, entirely fertile branches visible in the pustules. Rarely a faint coconut odor detected.

Distribution — Brazil, Canada, Cuba, Ethiopia, Ecuador, Germany, Ghana, Peru. Puerto Rico, U.S.A. (Samuels et al. 2006), Japan (Yabuki et al. 2014), and South Korea.

Specimens examined: **KOREA, Seoul,** Seongbuk-gu, Anam-dong, Korea University, chromated copper arsenate treated wood of *Pinus radiata*, 2003, Jae-Jin Kim (NIBR-FG0000120472, culture KUC3025, GenBank KJ652555); Mapo-gu, Sangam-dong, creosote contaminated soil, May 2007, Jae-Jin Kim (NIBR-FG0000120474, culture KUC5063, GenBank HM534662; NIBR-FG0000120475, culture KUC5064, GenBank KJ652554). **Gyeonggi-do,** Gwangmyeong-si, creosote treated wood, 6 November 2007, Jae-Jin Kim (NIBR-FG0000120473, culture KUC5028, GenBank KJ652556)

Remarks — The colony morphology and microscopic features of the Korean isolates agreed well with the protologue description, except for the protologue conidia being longer ((3.0–)3.5–4.5(–6.2) µm; Samuels et al. 2006).

The TEF sequences of *T. koningiopsis* KUC3025 (604 bp), KUC5028 (580 bp), KUC5063 (555 bp), and KUC5064 (553 bp) were placed inside the *T. koningiopsis* clade with high support (PP = 1). *Trichoderma koningiopsis* KUC5028, KUC5063 and KUC5064 were placed in the same subclade with high support (PP = 1). However, *T. koningiopsis* KUC3025 was clustered in another subclade with *T. koningiopsis* GJS 06-219 with high support (PP = 1). This intraspecific genetic variation was not associated with any phenotypic variation.

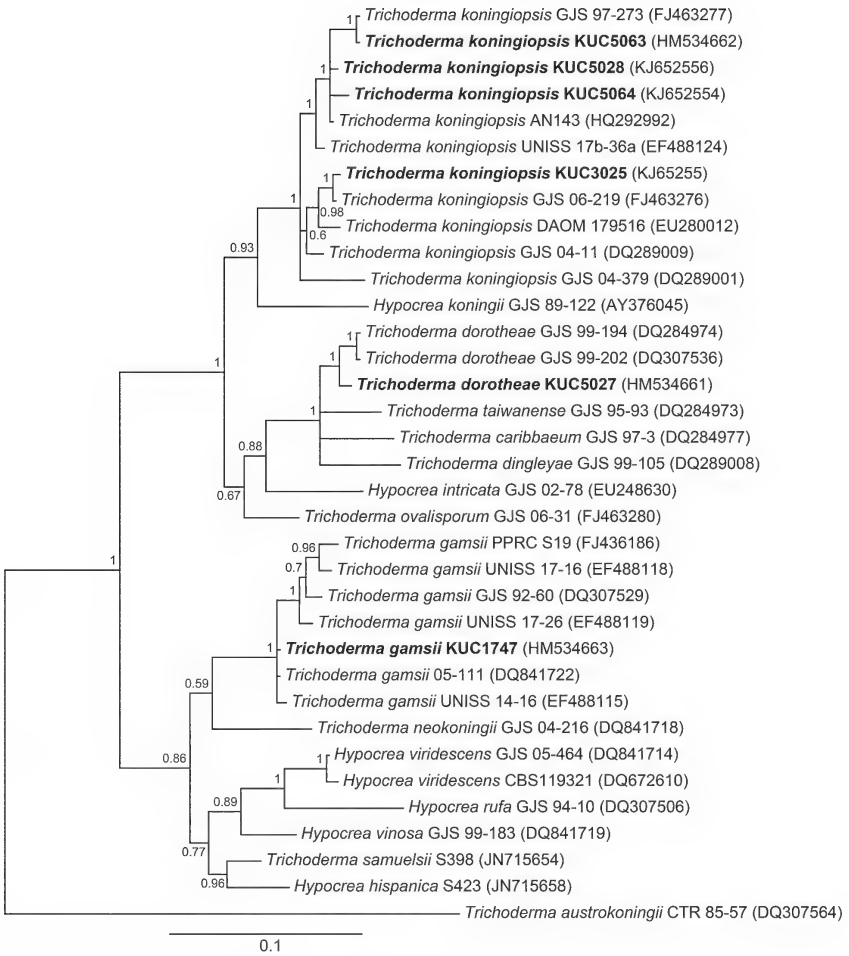
Trichoderma koningiopsis GJS 97-273 (FJ463277)
Trichoderma koningiopsis KUC5063 (HM534662)
Trichoderma koningiopsis KUC5028 (KJ652556)
Trichoderma koningiopsis KUC5064 (KJ652554)
Trichoderma koningiopsis AN143 (HQ292992)
Trichoderma koningiopsis UNISS 17b-36a (EF488124)
Trichoderma koningiopsis KUC3025 (KJ65255)
Trichoderma koningiopsis GJS 06-219 (FJ463276)
Trichoderma koningiopsis DAOM 179516 (EU280012)
Trichoderma koningiopsis GJS 04-11 (DQ289009)
Trichoderma koningiopsis GJS 04-379 (DQ289001)
Hypocrea koningii GJS 89-122 (AY376045)
Trichoderma dorotheae GJS 99-194 (DQ284974)
Trichoderma dorotheae GJS 99-202 (DQ307536)
Trichoderma dorotheae KUC5027 (HM534661)
Trichoderma taiwanense GJS 95-93 (DQ284973)
Trichoderma caribbaeum GJS 97-3 (DQ284977)
Trichoderma dingleyae GJS 99-105 (DQ289008)
Hypocrea intricata GJS 02-78 (EU248630)
Trichoderma ovalisporum GJS 06-31 (FJ463280)
Trichoderma gamsii PPRC S19 (FJ436186)
Trichoderma gamsii UNISS 17-16 (EF488118)
Trichoderma gamsii GJS 92-60 (DQ307529)
Trichoderma gamsii UNISS 17-26 (EF488119)
Trichoderma gamsii KUC1747 (HM534663)
Trichoderma gamsii 05-111 (DQ841722)
Trichoderma gamsii UNISS 14-16 (EF488115)
Trichoderma neokoningii GJS 04-216 (DQ841718)
Hypocrea viridescens GJS 05-464 (DQ841714)
Hypocrea viridescens CBS119321 (DQ672610)
Hypocrea rufa GJS 94-10 (DQ307506)
Hypocrea vinosa GJS 99-183 (DQ841719)
Trichoderma samuelsii S398 (JN715654)
Hypocrea hispanica S423 (JN715658)
Trichoderma austrokoningii CTR 85-57 (DQ307564)
0.1

FIG. 2. 50% majority-rule consensus tree of *Trichoderma dorotheae, T. gamsii, T. koningiopsis*, and allied species. Strains analyzed in this study are in bold font. GenBank accession numbers are in parentheses. Posterior probabilities ≥0.50 are shown above branches.

Even though *T. koningiopsis* caused green discoloration on wood surfaces, its wood-discoloration rate was low (Huh et al. 2011). *Trichoderma koningiopsis* KUC5063 showed strong antagonistic properties against wood-damaging fungi (Lee et al. 2012).

Phylogeny

The Bayesian tree of TEF region sequences was constructed to confirm the morphological identification. The aligned dataset of TEF contained 35 sequences and 600 characters. The three species are placed into Viride–Koningii section (Jaklitsch 2011).

Trichoderma gamsii was grouped with *T. neokoningii* Samuels & Soberanis (Fig. 2). Our phylogenetic result was similar to the study by Jaklitsch et al. (2006).

Trichoderma koningiopsis was monophyletic with *T. koningii* Oudem. [= *Hypocrea koningii* Lieckf. et al.], and *T. dorotheae* was placed in a monophyletic group with *T. caribbaeum* Samuels & Schroers, *T. dingleyae* Samuels & Dodd, and *T. taiwanense* Samuels & M.L. Wu. Again, the *T. koningiopsis* and *T. dorotheae* clades grouped together. However, phylogenetic trees in Jaklitsch (2011) showed that *T. koningiopsis* and *T. dorotheae* were monophyletic, but not with *T. koningii*. *Trichoderma koningii* was placed in the basal lineage below the two species (Jaklitsch 2011).

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Terriera fici sp. nov. on *Ficus vasculosa* from Hainan Province, China

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Abstract — *Terriera fici*, a new species that develops on leaves of *Ficus vasculosa*, was collected in 2014 from Jianfengling National Forest Park of Hainan Province, China. Description, illustration, and comments are given for this fungus. The type collection is deposited in the Reference Collection of Forest Fungi of Anhui Agricultural University, China (AAUF).

Key words — *Rhytismataceae, Rhytismatales, Moraceae*, morphology, taxonomy

Introduction

Eriksson (1970) erected the monotypic genus *Terriera* B. Erikss. for *T. cladophila* (Lév.) B. Erikss.; Minter (1996) provided a detailed description of the type species. *Terriera* species are distributed worldwide and associate with both angiosperms and gymnosperms. IndexFungorum (2015) lists 27 specific and two varietal epithets for the genus, of which SpeciesFungorum (2015) accepts 24 species in *Terriera*.

Recently, during investigations of the *Rhytismatales* from Jianfengling National Forest Park of Hainan Province, China, a new *Terriera* species was discovered.

Materials & methods

The study was based on collections made in 2014 from Hainan, China. Specimens were rehydrated in water for 15 min and ascomata and conidiomata were sliced into 10–15 µm thick sections using a freezing microtome (Jinhua, China). The structure of

[A] Yuan Wu and Shi-Juan Wang contributed equally to this work.

the fruiting bodies was observed in 0.1% (w/v) cotton blue in phenol glycerin. The color of the internal structures of the fruiting bodies was observed in water. Measurements and drawings of asci, ascospores, paraphyses, conidiogenous cells and conidia were made from squash mounts in 5% KOH solution. Line and point integrated illustrations of internal structures of ascomata were prepared using a Panasonic XSJ-2 microscope drawing device. Photographs were taken using a Sony DSC-TX7C camera and a digital camera connected to an Olympus CX31RTSF microscope. The type specimen is deposited in the Reference Collection of Forest Fungi of Anhui Agricultural University, Hefei, China (AAUF).

Taxonomy

Terriera fici Y.R. Lin & Yuan Wu, **sp. nov.** FIGS 1, 2

MycoBank MB812746

Differs from *Terriera nitens* by its larger subrounded ascomata, its much thinner excipulum, its well developed subhymenium consisting of textura angularis and intricata, its septate paraphyses branching 1–2 times, its somewhat long-stalked asci, and the absence of trichogynes in its conidiomata.

TYPE: China, Hainan, Jianfengling National Park, Tianchi, alt. 880 m, on dead leaves of *Ficus vasculosa* Wall. ex Miq. (*Moraceae*), 18 June 2014, Y.R. Lin, S.J. Wang & Y.F. Xu 2804 (**Holotype**, AAUF 68912).

ETYMOLOGY: *fici*, referring to the host genus.

COLONIES on both sides of dead leaves, forming distinct subround to irregular, yellowish to gray-white bleached spots 5–12 mm diam. that tend to coalesce into larger irregular shapes.

ZONE LINES somewhat frequent, gray-black or black, thin, surrounding or partly surrounding the pale areas.

CONIDIOMATA developing on both sides of fallen leaves, principally on the lower side of the leaf, scattered, occasionally coalescent. In surface view, conidiomata 90–200 µm diam., rounded or subrounded, somewhat raising the substratum surface, dark brown to gray-black, discharging spores through an conspicuous central ostiole or split. In vertical section, conidiomata subepidermal, lenticular. UPPER WALL 10–18 µm thick, black-brown but roughly black near the opening, consisting of 1.5–4 µm diam., thick-walled angular cells. BASAL WALL dark brown, 10–20 µm thick, composed of 2–3 µm diam. angular cells. SUBCONIDIOGENOUS LAYER 4–8 µm thick, comprising nearly colorless, thin-walled angular cells. CONIDIOGENOUS CELLS 9–14 × 2–3 µm, subcylindrical, tapering towards the apex, proliferating sympodially and percurrently. CONIDIA 4–5 × 1–1.2 µm, cylindrical or subclavate, hyaline, straight or slightly curved.

ASCOMATA in similar positions to conidiomata on the substratum, scattered to clustered. In surface view, ascomata 360–520 µm diam., rounded or

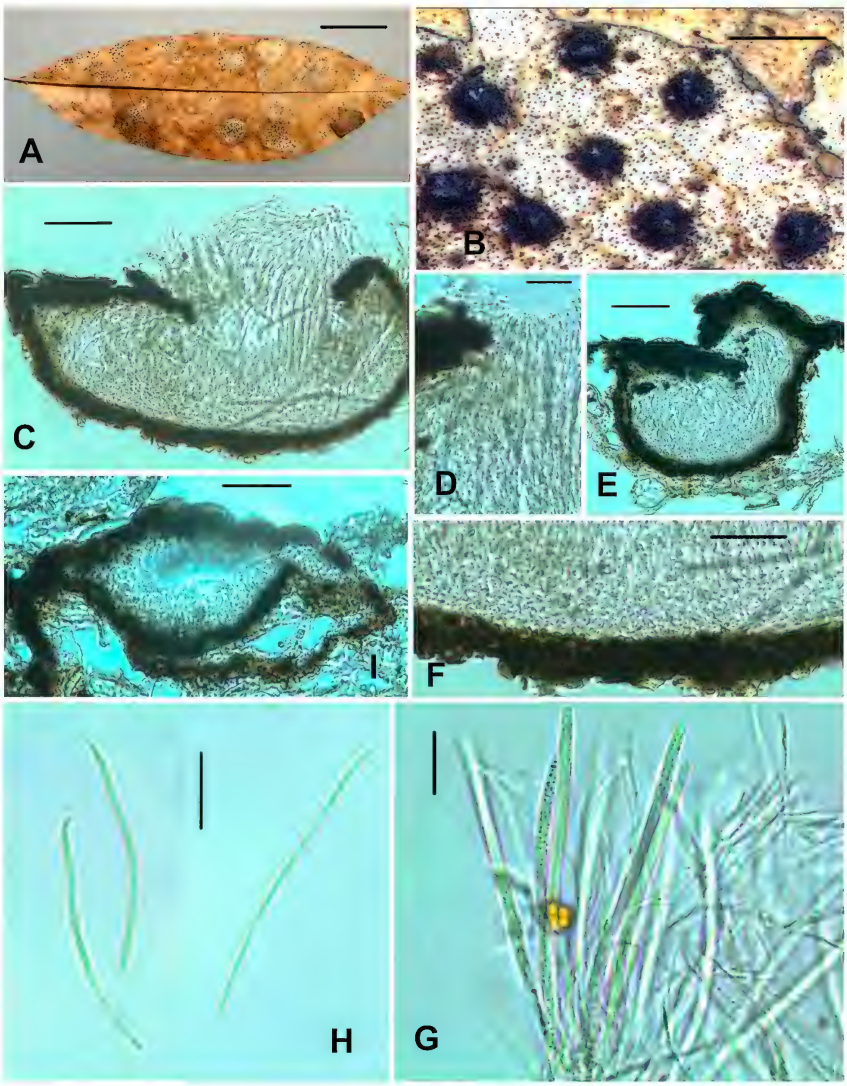

Fig. 1. *Terriera fici* (holotype AAUF 68912) on *Ficus vasculosa:* A. Fruiting bodies on leaf. B. Detail of ascomata and conidiomata. C. Ascoma in median vertical section. D. Upper portion of hymenium. E. Ascoma in section, showing strongly carbonized and brittle tissue of covering stromatic extension. F. Subhymenium and basal stroma. G. Asci and paraphyses. H. Ascospores. I. Conidioma in median vertical section. Scale bars: A = 20 mm; B = 8 mm; C = 50 µm; D, H = 20 µm; E, F, I = 30 µm; G = 15 µm.

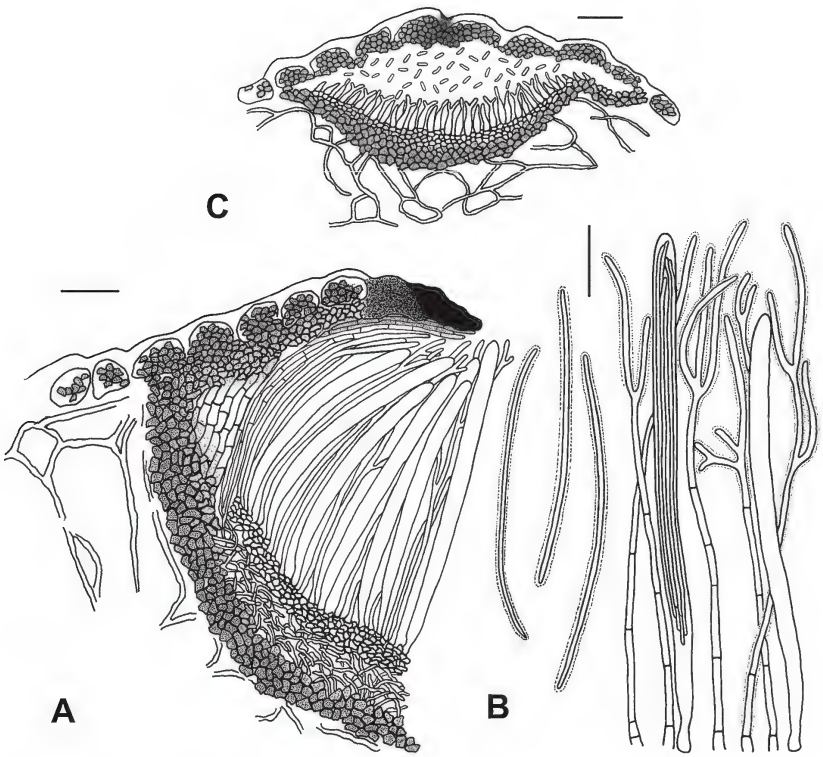

Fig. 2. *Terriera fici* (ex holotype AAUF 68912): A. Portion of ascoma in median vertical section. B. Asci, paraphyses, and ascospores. C. Conidioma in median vertical section. Scale bars: A = 20 µm; B = 15 µm; C = 10 µm.

subrounded, black, slightly shiny, with conspicuous edge, moderately raising the surface of the leaf, opening by a single longitudinal split extending 2/3–4/5 the length of the ascoma. In median vertical section, ascomata subepidermal. Covering stroma 18–25 µm thick, composed of a black-brown textura angularis with thick-walled cells 3–5.5 µm diam., connecting to the basal stroma. Along the edge of the ascomatal opening, there is a 8–15 µm thick, flat extension adjacent to the covering stroma, which is comprised of strongly carbonized and brittle tissue with no obvious cellular structure. Excipulum very poorly developed, arising from the inner layer of the basal stroma, consisting of hyaline textura porrecta. Basal stroma 15–22 µm thick, dark brown to black, composed of (3–)4–6 rows of 3.5–6 µm diam., angular, thick-

walled cells. A layer of 10–30 µm thick textura prismatica, comprised of nearly colorless or light brown cells 2.5–4 µm diam. occurs between the covering and basal stromata. Subhymenium well developed, 15–20 µm thick, strongly concave to form a cup shape, consisting of hyaline textura angularis and intricata. Paraphyses 100–120 × 1–1.2 µm, filiform, septate, mostly branching 1–2 times in the upper portion, sometimes slightly tapered at the branched apices, covered in a c. 1 µm thick gelatinous matrix. Asci ripening sequentially, 90–115 × 4–5.5 µm, narrow cylindrical, apex rounded or subacute, without circumapical thickening, not bluing in iodine, discharging spores through a small apical pore, 8-spored, stalk 20–25 µm in length. Ascospores arranged in a fascicle, 65–80 × 0.8–1 µm, filiform, colorless, aseptate, rounded to obtuse at the apex, slightly tapered towards the rounded or subacute base, hyaline, with a c. 0.5 µm thick gelatinous sheath.

Host, habitat, & distribution: Producing conidiomata and ascomata on fallen leaves of *Ficus vasculosa*. Known only from the type locality, Hainan Province, China.

Comments: *Terriera fici* is very similar to *T. nitens* Y.R. Lin in the ascomatal openings, the appearance of the conidiomata, and the ascal and ascospore shapes. However, *T. nitens* differs in its suborbicular or broadly elliptical smaller ascomata (240–320 × 190–250 µm), a well developed excipulum (12–16 µm thick near the opening), a much thinner subhymenium (5–9 µm) consisting of textura porrecta, unbranched aseptate paraphyses, slightly short-stalked asci, and trichogynes present in the conidiomata (Chen et al. 2014).

Terriera minor (Tehon) P.R. Johnst. is distinguished from the new species by the absence of zone lines and asexual conidiomata, oblong to oblong-elliptic ascomata, a flat or slightly concave subhymenium, much wider asci (6–7 µm), paraphyses extending 20–30 µm beyond asci, and slightly larger ascospores (70–100 × 1.5–2 µm) (Johnston 1989, as *Lophodermium minus*).

Terriera fici also resembles *Lophodermium illiciicola* S.J. Wang et al., which is distinguished by smaller ascomata (280–360 × 260–300 µm), absent conidiomata, a thinner subhymenium (10–15 µm thick) consisting of textura angularis-porrecta, and unbranched paraphyses markedly longer than the asci (Wang et al. 2006).

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Six new *Russula* records from Turkey

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Abstract — During field studies in 2012–13, six *Russula* species from the Samanlı Mountains region were identified as new records for Turkey: *R. clariana*, *R. faginea*, *R. insignis*, *R. odorata*, *R. sericatula*, and *R. velutipes*. Descriptions of the taxa along with their distinct features are given and a key to the species is provided.

Key words — *Basidiomycota*, *Russulaceae*, Turkish mycobiota

Introduction

There have been many studies of the diversity of macrofungi in Turkey. Sesli & Denchev (2008) noted that 2158 macrofungi species were recorded for Turkish mycota. Subsequent studies by Sesli & Kobayashi (2014), Güngör et al. (2014), Solak et. al (2014a,b), Sesli (2014), Uzun et al. (2014), and Akata et al. (2014) have increased that number to 2191. Here we record six additional *Russula* spp. collected in Samanlı Mountains, Turkey

Samanlı Mountains lie southeast of the Marmara Region and stretch between Bozburun at the edge of Armutlu Peninsula in the west and Geyve Strait formed by Sakarya River in the east. A close look on the natural vegetation of the area highlights kermes oak (*Quercus coccifera* L.), holly oak (*Quercus ilex* L.), and bay laurel (*Laurus nobilis* L.) as the main shrubs and ligneous plants in the maquis formation of Samanlı Mountains up to 500–600 m. Hawthorn (*Crataegus oxyacantha* L.) and boxwood (*Buxus sempervirens* L.) are also seen in patches among maquis elements. Main ligneous plants in the forest cover of the area are pedunculate oak (*Quercus robur* L.), oriental beech (*Fagus orientalis* Lipsky), Uludağ fir (*Abies bornmuelleriana* Mattf.), chestnut (*Castanea sativa* Mill.), black pine (*Pinus nigra* subsp. *pallasiana* (Lamb.) Holmboe, stone pine (*Pinus pinea* L.), Turkish pine (*Pinus brutia* Ten.), common hornbeam

(*Carpinus betulus* L.), and Scots pine (*Pinus sylvestris* L.). Also seen in patches are groups of oriental plane (*Platanus orientalis* L.), maple (*Acer platanoides* L.), and white poplar (*Populus alba* L.). The area is located in the Mediterranean climatic zone, and the annual rainfall varies between 400mm and 1200mm in different regions (Akman, 1999).

Materials & methods

The macrofungi were collected in the provinces Bursa, Yalova, Kocaeli during 2012–13. Important macroscopical features and ecological information were noted in the field, and digital images were taken of the specimens in their habitat. Collected specimens dried in dehydrators after each study day; the dried materials were put into plastic bags to bring them to the fungarium in good condition for further analysis.

Micromorphological characters were examined using a Leica DM3000 light microscope and photographed digitally. Specimen tissues were examined in Melzer's reagent, 5% KOH, and SBA (sulfobenzaldehyde), and the pileus context was spot-tested with $FeSO_4$ and phenol. At least 20 spores per specimen were measured. The specimens were identified according to Galli (2003), Sarnari (1998, 2006), and Kränzlin (2005). The specimens are kept in the Fungarium of Selçuk University, Konya, Turkey (SELUK).

Taxonomy

Russula clariana Kuyper & Vuure, Persoonia 12: 452. 1985. FIGS 1A, 2A

PILEUS 40–70 mm across, hemispherical when young, later plane, violet, purple, usually light olive color in the center, margin short striate. LAMELLAE white, narrowly attached. STIPE 30–50 × 10–20 mm, cylindrical to slightly clavate, white. SPORES 7–8.5 × 6–7.5 µm, subglobose, with solitary warts. BASIDIA 35–55 × 10–15 µm, clavate, with 4 sterigmata. CHEILOCYSTIDIA and PLEUROCYSTIDIA 45–85 × 10–15 µm, fusiform to clavate, with gray-black contents in SBA.

MACROCHEMICALS: $FeSO_4$ negative, phenol light rose.

SPECIMENS EXAMINED: **TURKEY, BURSA**: Mahmudiye, Taşlıtarla, 40°36′09″N 29°46′04″E, 947 m, on soil under *Fagus orientalis*, 4 Jun 2013, leg. H.H. Doğan & Ö. Öztürk, HHD 10308, 10319, 10453 (SELUK).

Russula faginea Romagn., Russules Eur. Afr. Nord: 681. 1967. FIGS 1B, 2B,C

PILEUS 40–80 mm across, convex to plane, wine-red to pink, usually whitish yellow tones in the center. LAMELLAE white, narrowly attached. STIPE 30–70 × 10–25 mm, cylindrical, white when young, later yellowing to browning. SPORES 7–10 × 6–8.5 µm, subglobose to elliptical, with distant warts. BASIDIA 45–60 × 10–15 µm, clavate, with 4 sterigmata. CHEILOCYSTIDIA and PLEUROCYSTIDIA 45–85 × 11–14 µm, fusiform, obtuse or with a prominent projection, with gray-black contents in SBA.

Figure 1. Basidiocarps: A, *Russula clariana*; B, *R. faginea*; C, *R. insignis*; D, *R. odorata*; E, *R. sericatula*; F, *R. velutipes*.

Macrochemicals: $FeSO_4$ green, phenol reddish brown.

Specimen examined: **TURKEY**, **Yalova**: Çanakpınar, 40°32′40″N 29°11′34″E, 700 m, on soil under *Fagus orientalis*, 7 Jun 2013, leg. H.H. Doğan & Ö. Öztürk HHD10509 (SELUK).

Russula insignis Quél., C.R. Ass. Franç. Av. Sci. 16: 588. 1888. FIGS 1C, 3A,B

PILEUS 40–60 mm across, plane, funnel-shaped later, dark grey-brown to brown. LAMELLAE white, turning yellow or brown later, notched. STIPE 30–50 × 10–15 mm, cylindrical, white, turning yellow with age. SPORES 6.5–8.5 × 5–6.5 µm, subglobose to elliptical, with elongated warts. BASIDIA 40–50 × 8–15 µm, clavate, with 4 sterigmata. CHEILOCYSTIDIA and PLEUROCYSTIDIA 40–80 × 6–15 µm, clavate to fusiform, with faintly gray- black contents in SBA.

MACROCHEMICALS: $FeSO_4$ pale pink, phenol wine red.

SPECIMENS EXAMINED: **TURKEY, BURSA**: Gemlik, Haydariye, District No. 250, 40°30′27″N 29°09′44″E, 740 m, on soil under *Quercus coccifera* and *Fagus orientalis*, 3 Jun 2013, leg. H.H. Doğan & Ö. Öztürk HHD10239, 10241 (SELUK).

Russula odorata Romagn., Bull. Soc. Linn. Lyon 19: 76. 1950. FIGS 1D, 2D–F

PILEUS 30–60 mm across, plane, carmine to light red, entire pileus often strongly fading to white. LAMELLAE whitish when young after pale yellow, narrowly attached. STIPE 25–50 × 5–10 mm, cylindrical, white and somewhat yellowing from the base upward. SPORES 6.5–8.5 × 5.5–7 µm, subglobose to elliptical, with connected warts. BASIDIA 35–45 × 10–15 µm, clavate. CHEILOCYSTIDIA and PLEUROCYSTIDIA 45–75 × 10–15 µm, fusiform, apically with a small knob, with gray-black contents in SBA.

MACROCHEMICALS: $FeSO_4$ pink, phenol wine brown.

SPECIMEN EXAMINED: **TURKEY, SAKARYA**: Akyazı, near Keremali Forester Cottage, 40°39′10″N 30°43′38″E, 972 m, on soil under *Fagus orientalis*, 11 Nov 2012, leg. H.H. Doğan & Ö. Öztürk HHD8660 (SELUK).

Russula sericatula Romagn., Bull. Soc. Linn. Lyon 27: 287. 1958. FIGS 1E, 3F,G

PILEUS 50–80 mm across, plane, later infundibuliform, violet-brown, cinnamon-brown. LAMELLAE white, narrowly attached. STIPE 50–70 × 10–20 mm, cylindrical, white, turning yellow or brown with age. SPORES 7–9.5 × 6–8 µm, subglobose, with elongated warts. BASIDIA clavate, 40–50 × 10–15 µm, with 4 sterigmata. CHEILOCYSTIDIA and PLEUROCYSTIDIA 50–70 × 10–15 µm, fusiform, most with a small apical projection, with gray-black contents in SBA.

MACROCHEMICALS:: $FeSO_4$ light pink, phenol wine brown.

SPECIMEN EXAMINED: **TURKEY, KOCAELI**: Gölcük, Menekşe Yaylası, District No: 13, 40°35′01″N 29°54′48″E, 890 m, on soil under *Fagus orientalis*, 5 Jun 2013, leg. H.H. Doğan & Ö. Öztürk HHD10398 (SELUK).

Russula velutipes Velen., České Houby 1: 133. 1920. FIGS 1F, 3C–E

PILEUS 30–70 mm, convex to plane, pink to red, yellowish toward to center. LAMELLAE white when young, later pale cream-yellow, narrowly attached. STIPE 40–60 × 10–25 mm, clavate, white. SPORES 6.5–8 × 5–6.5 µm, elliptical,

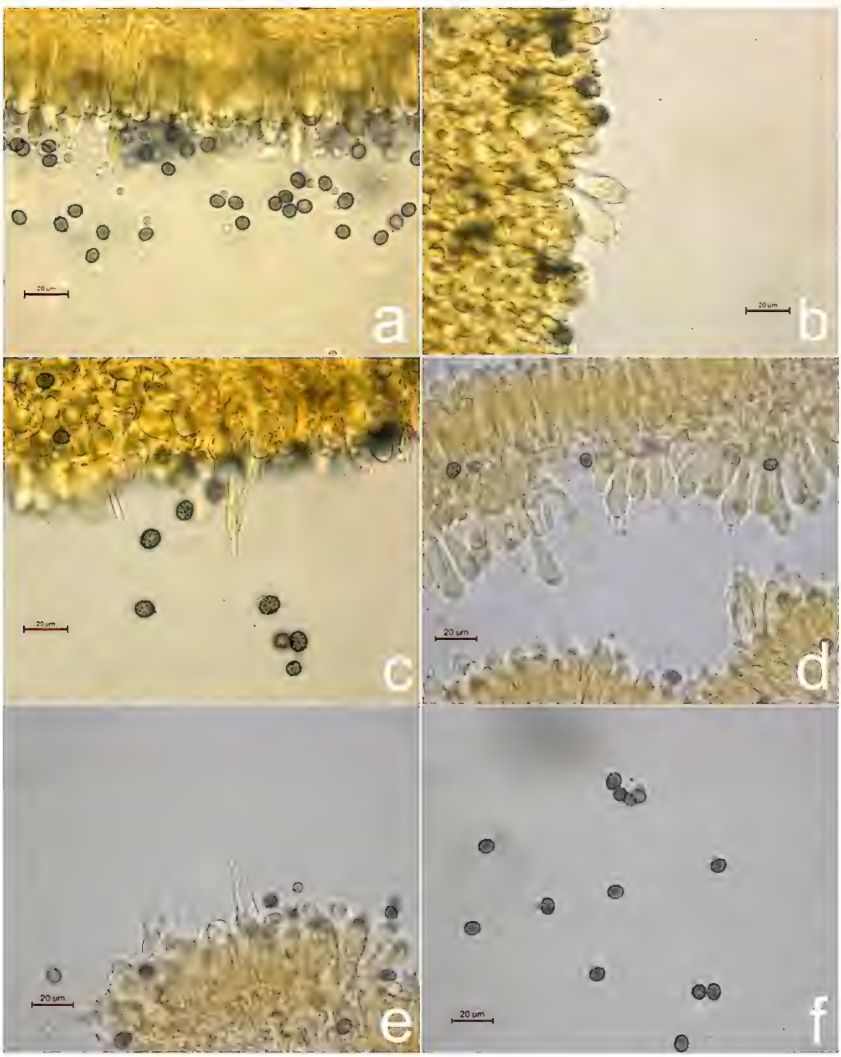

FIGURE 2. ***Russula clariana***: A, pleurocystidia and basidiospores. ***R. faginea***: B, basidia; C, pleurocystidia and basidiospores. ***R. odorata***: D, basidia;. E, cheilocystidia; F, basidiospores.

with elongated warts. BASIDIA 35–50 × 5–10 µm, clavate, with (2–)4 sterigmata. CHEILOCYSTIDIA 40–70 × 5–15 µm, fusiform, most obtuse. PLEUROCYSTIDIA 40–70 × 5–15 µm, fusiform most with an apical projection which is sometimes forked, with faintly gray-black contents in SBA.

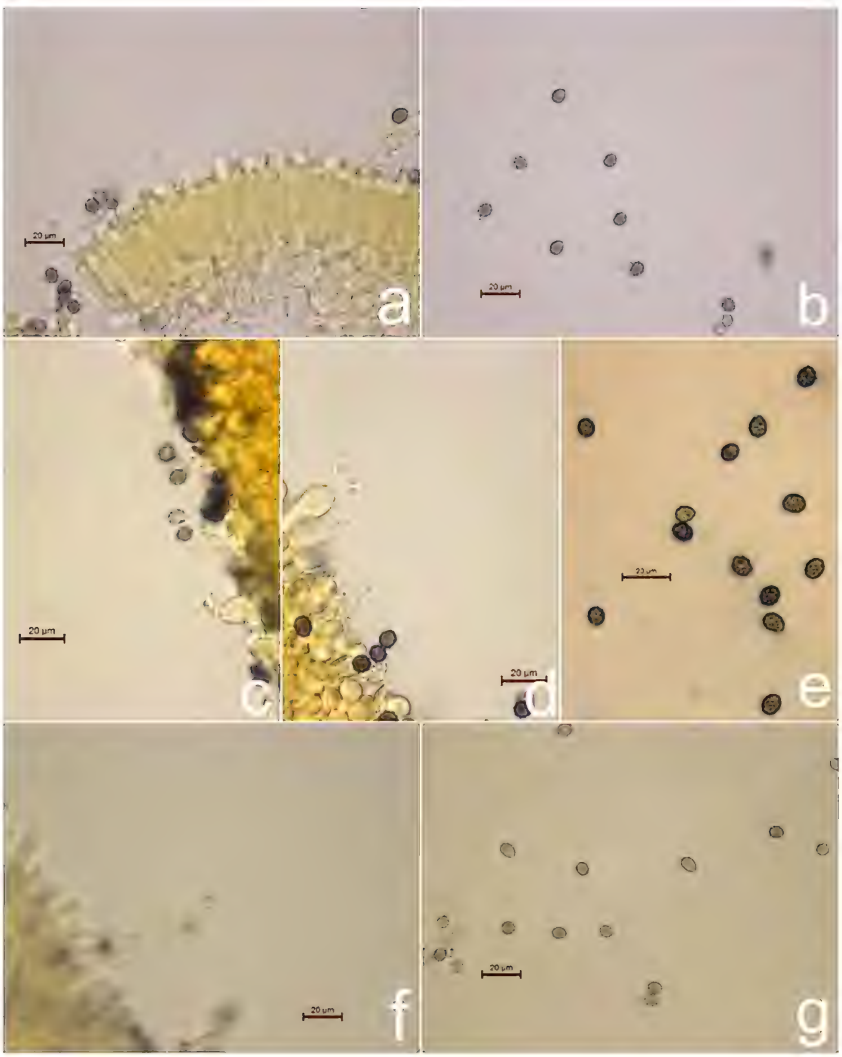

FIGURE 3. ***Russula insignis***: A, pleurocystidia; B, basidiospores. ***R. velutipes***: C, basidia (2-4 sterigmata); D, cheilocystidia; E, basidiospores. ***R. sericatula***: F, pleurocystidium; G, basidiospores.

MACROCHEMICALS: $FeSO_4$ pink, phenol purple brown.

SPECIMEN EXAMINED: **TURKEY**, **BURSA**: Mahmudiye, Taşlıyayla, 40°36′09″N 29°46′04″E, 947 m, on soil under *Fagus orientalis*, 4 Jun 2013, leg. H.H. Doğan & Ö. Öztürk HHD10314 (SELUK).

Discussion

Sesli & Denchev (2008) cite 108 *Russula* taxa as previously recorded for the Turkish mycobiota; six additional species are recorded here for the first time. The examined specimens do not significantly differ morphologically from the species descriptions in the literature. The simple key to the six identified species indicates the diagnostic differences among the species.

1. $FeSO_4$ reaction negative ..... *R. clariana*
   $FeSO_4$ reaction positive 2
2 $FeSO_4$ reaction light to dark green ..... *R. faginea*
   $FeSO_4$ reaction light pink to pink 3
3. Pileus rose, light pink, entire pileus whitening with age ..... *R. odorata*
   Entire pileus not whitening with age 4
4. Phenol reaction wine-red ..... *R. insignis*
   Phenol reaction not wine-red 5
5. Pileus purple-brown ..... *R. sericatula*
   Pileus pink to red ..... *R. velutipes*

Russula odorata has typically fruity odor and the pileus of our specimen was almost white, as it was old. *Russula insignis* is known as obligately mycorrhizal with *Quercus* species, and we did collect our specimen in an oak forest. A mycorrhizal association with *Fagus* and 2- to 4-spored basidia the important diagnostic characters for *R. velutipes*, which we observed in our specimen. *Russula faginea* can be easily distinguished with its dark green $FeSO_4$ reaction. In contrast, *R. clariana* is typical with its negative $FeSO_4$ reaction. *Russula sericatula* can be distinguished from the other species by its larger spores and a pileus that yellows toward the disc.

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Dictyosporium amoenum sp. nov. from Chapada Diamantina, Bahia, Brazil

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Abstract — *Dictyosporium amoenum*, a new asexual fungus collected on leaves of *Calophyllum brasiliense* in the Brazilian semiarid Caatinga region, is described and illustrated. It is distinguished by digitate complanate brown conidia composed of four parallel compact rows of cells, each row with a globose hyaline mucous apical appendage. Conidial illustrations and a table comparing all *Dictyosporium* species are provided.

Key words — conidial fungi, leaf litter, taxonomy

Introduction

During several mycological surveys of conidial fungi on decaying leaves of *Calophyllum brasiliense* in the Chapada Diamantina, Bahia, Brazil, a conspicuous fungus was collected that is clearly related to *Dictyosporium* Corda, introduced by Corda (1836) with *D. elegans* Corda as the type species. The genus comprises 47 accepted species distinguished by conidiomata that are sporodochial, scattered, compact pulvinate, and olivaceous to dark brown. Conidiophores are inconspicuous and the conidiogenous cells are monoblastic, discrete, cylindrical, doliiform or inflated, and determinate. Conidial secession is rhexolytic. The conidia are solitary, digitate, complanate to complex subcylindrical, multiseptate and comprising several rows of cells that are brown, pale brown or yellowish brown, and sometimes with mucilaginous,

clavate, globose, filiform to tubular-capitate, hyaline appendages or tunica at the apex of or (rarely) lateral to some or all rows of cells.

Materials & methods

Samples of leaves of *C. brasiliense* were placed in paper bags, taken to the laboratory, and treated according to Castañeda-Ruiz (2005). Mounts were prepared in PVL (polyvinyl alcohol and lactic acid) and measurements were taken at ×1000. Micrographs were obtained with an Olympus microscope (model BX51) equipped with bright field and Nomarski interference optics. The holotype is deposited in the Herbarium of Universidade Estadual de Feira de Santana, Bahia, Brazil (HUEFS).

Taxonomy

Dictyosporium amoenum C.R. Silva, Gusmão & R.F. Castañeda, **sp. nov.** FIG. 1

MycoBank MB812865

Differs from all other *Dictyosporium* spp. by its conidia comprising four vertical rows of cells, each row with a globose mucilaginous hyaline apical appendage.

TYPE: Brazil, Bahia State, Piatã, Chapada Diamantina, Serra da Tromba, 13°05′S 41°51′W, alt. 1263 m, on decaying leaves of *Calophyllum brasiliense* Cambess. (*Calophyllaceae*), 31.VII.2014, coll. C.R. Silva (**Holotype**, HUEFS 211336).

ETYMOLOGY: Latin, *amoenus*, meaning pleasant, delightful, agreeable.

CONIDIOMATA on the natural substrate sporodochial, pulvinate, scattered, mucilaginous, pale brown. CONIDIOPHORES inconspicuous. CONIDIOGENOUS CELLS monoblastic, cylindrical, discrete determinate. CONIDIA solitary, digitate, complanate, 20–30 × 12.5–15 µm, smooth, brown, composed of four rows of cells compacted together, parallel, 5–7-septate, slightly constricted at septa, smooth, brown, all with a globose, mucilaginous, hyaline apical appendage, 10–17.5 µm wide

NOTE: Among 47 described *Dictyosporium* species, only seventeen have been recorded with conidial appendages: *D. alatum* Emden, *D. aquaticum* Abdel-Aziz, *D. bulbosum* Tzean & J.L. Chen, *D. canisporum* L. Cai & K.D. Hyde, *D. digitatum* J.L. Chen et al., *D. freycinetiae* McKenzie, *D. gauntii* Bhat & B. Sutton, *D. hughesii* McKenzie, *D. inflatum* (Matsush.) K.L. Pang et al., *D. manglietiae* Kodsueb & McKenzie, *D. musae* Photita, *D. nigroapice* Goh et al., *D. strelitziae* Crous & A.R. Wood, *D. tetraploides* L. Cai & K.D. Hyde, *D. tetraseriale* Goh et al., *D. thailandicum* M.J. D'souza et al., and *D. zhejiangense* Wongs. et al. All these taxa differ from *D. amoenum* in the number of rows of cells and in the number, shape, and size of apical appendages on their conidia. (TABLE 1, FIGS 2–4; data were obtained and conidial drawings adapted from Arambarri et al. 2001, Cai & Hyde 2007, Cai et al. 2003a,b; Castañeda-Ruiz et al. 1997; Crous et al. 2009; Crous et al. 2011, Goh et al. 1999; Hu et al. 2010,

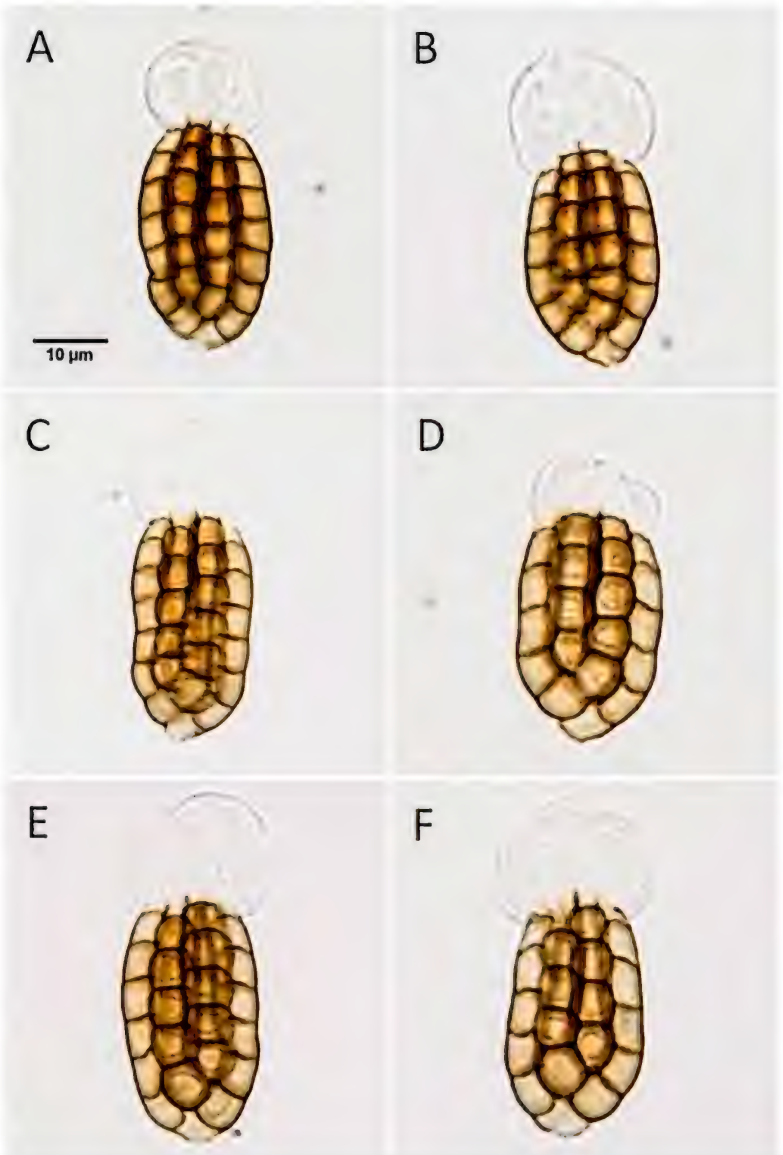

Fig. 1. *Dictyosporium amoenum* (ex HUEFS 211336). Conidia.

Liu et al. 2015; Kirschner et al. 2013; Kodsueb et al. 2006; McKenzie 2008, 2010; and Photita et al. 2002, Whitton et al. 2012, Wongsawas et al. 2009, Zhang 2009, and Zhao & Zhang 2003).

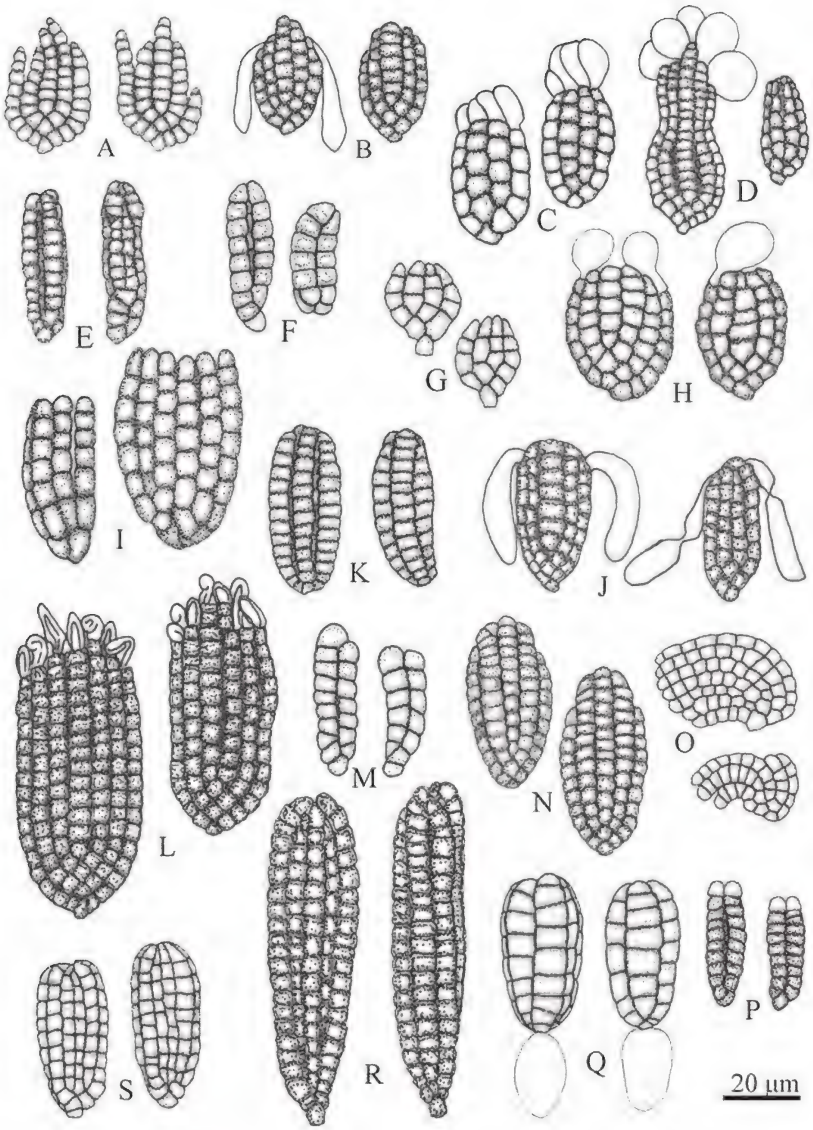

Fig. 2. Conidia of *Dictyosporium* species: **A**. *D. acroinflatum* (Whitton et al. 2012); **B**. *D. alatum* (Goh et al. 1999); **C**. *D. amoenum* (this paper); **D**. *D. aquaticum* (Liu et al. 2015); **E**. *D. australiense* (Goh et al. 1999); **F**. *D. biseriale* (Hu et al. 2010); **G**. *D. brahmaswaroopii* (Goh et al. 1999); **H**. *D. bulbosum* (Castañeda-Ruiz et al. 1997); **I**. *D. campaniforme* (Goh et al. 1999); **J**. *D. canisporum* (Cai et al. 2003a); **K**. *D. cocophilum* (Goh et al. 1999); **L**. *D. digitatum* (Goh et al. 1999); **M**. *D. dkagarwalii* (Manoharachary et al. 2007); **N**. *D. elegans* (Goh et al. 1999); **O**. *D. foliicola* (Goh et al. 1999); **P**. *D. freycinetiae* (McKenzie 2008); **Q**. *D. gauntii* (Goh et al. 1999); **R**. *D. giganticum* (Goh et al. 1999); **S**. *D. heptasporum* (Goh et al. 1999).

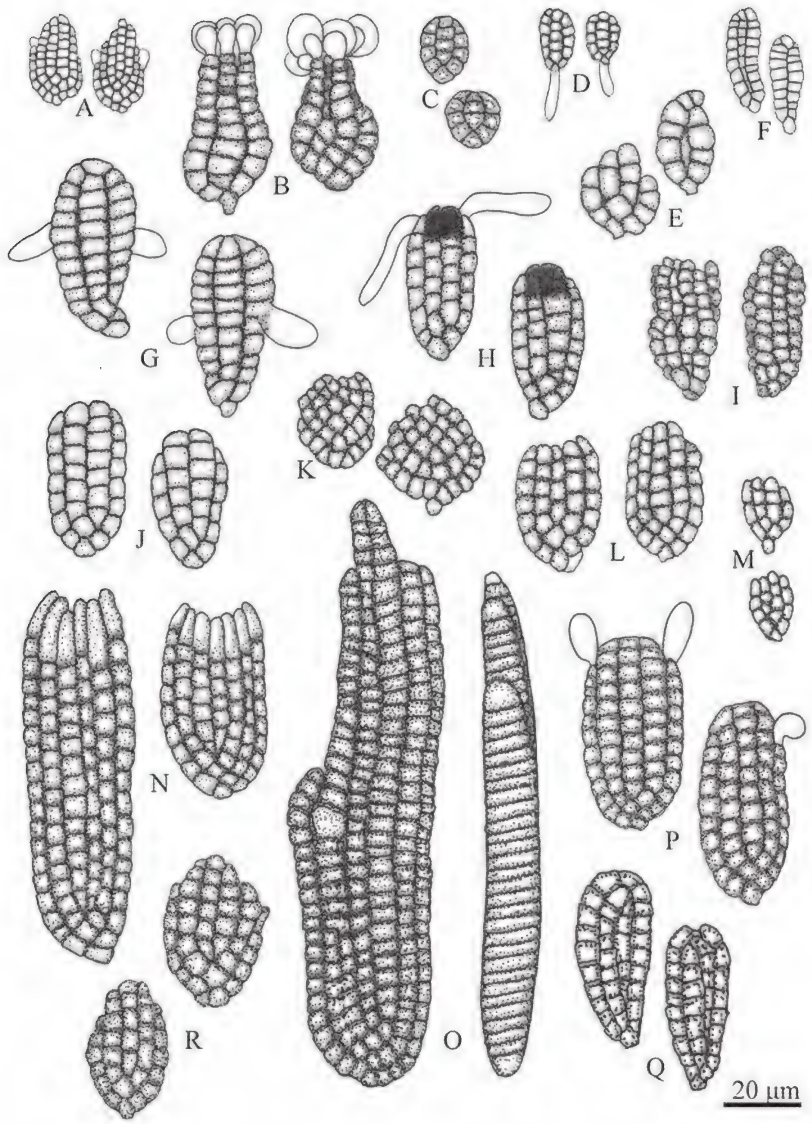

FIG. 3. Conidia of *Dictyosporium* species: **A**. *D. hughesii* (McKenzie 2010); **B**. *D. inflatum* (Kirschner et al. 2013); **C**. *D. lakefuxianense* (Cai et al. 2003b); **D**. *D. manglietiae* (Kodsueb et al. 2006); **E**. *D. meiosporum* (Liu et al. 2015); **F**. *D. micronesiacum* (Goh et al. 1999); **G**. *D. musae* (Photita et al. 2002); **H**. *D. nigroapice* (Goh et al. 1999); **I**. *D. oblongum* (Goh et al. 1999); **J**. *D. pandani* (Whitton et al. 2012); **K**. *D. polystichum* (Goh et al. 1999); **L**. *D. rhopalostylidis* (McKenzie 2010); **M**. *D. schizostachyfolium* (Goh et al. 1999); **N**. *D. sinensis* (Zhang 2009); **O**. *D. stellatum* (Crous et al 2011); **P**. *D. strelitziae* (Crous et al 2009); **Q**. *D. subramanianii* (Goh et al. 1999); **R** *D. taishanense* (Zhao & Zhang 2003).

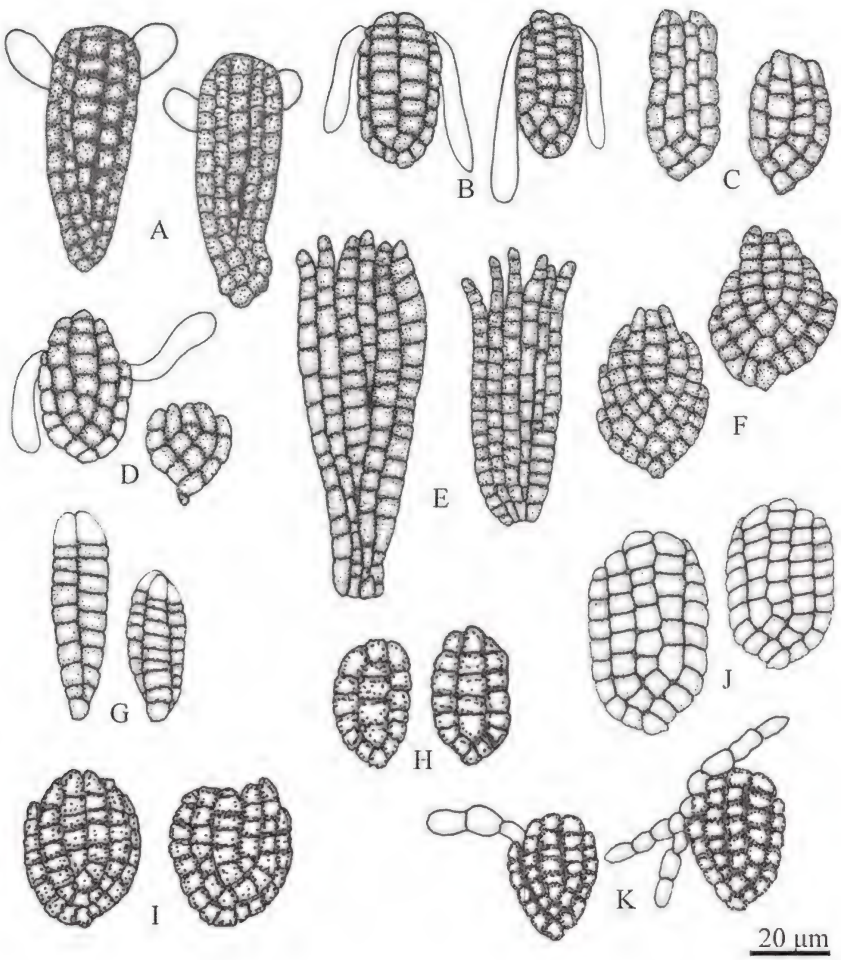

Fig. 4. Conidia of *Dictyosporium* species: **A**. *D. tetraploides* (Cai et al. 2003a); **B**. *D. tetraseriale* (Goh et al. 1999); **C**. *D. tetrasporum* (Cai & Hyde 2007); **D**. *D. thailandicum* (Liu et al. 2015); **E**. *D. tnlakhanpalii* (Manoharachary et al. 2007); **F**. *D. toruloides* (Goh et al. 1999); **G**. *D. triramosum* (Arambarri et al. 2001); **H**. *D. triseriale* (Goh et al. 1999); **I**. *D. yunnanense* (Cai et al. 2003b); **J**. *D. zeylanicum* (Goh et al. 1999); **K**. *D. zhejiangense* (Wongsawas et al. 2009).

Table 1. Comparison of conidia in *Dictyosporium* species

Species	Conidial size (μm)	No. of rows	Reference
D. amoenum	20–30 × 12–15	4	this paper
D. acroinflatum	28–52 × 23–35	4–8	Whitton et al. (2012)
D. alatum	26–32 × 15–25	5	Goh et al. (1999)
D. aquaticum	60–85 × 20–30	5–6	Liu et al. (2015)
D. australiense	36–43 × 11–12	3	Goh et al. (1999)
D. biseriale	22–30 × 9–10	2	Hu et al. (2010)
D. brahmaswaroopii	17–24 × 12.5–19	3–5	Goh et al. (1999)
D. bulbosum	27–46 × 11–30	5(–6)	Castañeda-Ruiz et al. (1997)
D. campaniforme	22–40 × 20–30	4–8	Goh et al. (1999)
D. canisporum	27–47 × 20–25	4–5	Cai et al. (2003a)
D. cocophilum	53–76 × 19–22	7	Goh et al. (1999)
D. digitatum	47–77 × 22–39	5–7	Goh et al. (1999)
D. dkagarwalii	30–40 × 9.5–11.5	2–3	Manoharachary et al. (2007)
D. elegans	40–80 × 24–31	5	Goh et al. (1999)
D. foliicola	34–56 × 20–38	5–7	Goh et al. (1999)
D. freycinetiae	27–40 × 5–6	3	McKenzie (2008)
D. gauntii	40–50 × 18–25	4–6	Goh et al. (1999)
D. giganticum	105–121 × 25–32	7	Goh et al. (1999)
D. heptasporum	50–80 × 20–30	7	Goh et al. (1999)
D. hughesii	33–50 × 23–32	7	McKenzie (2010)
D. inflatum	20–50×5–8(–10)	2–5	Kirschner et al. (2013)
D. lakefuxianense	15–22× 10–16.5	3	Cai et al. (2003b)
D. manglietiae	22–28 × 12.5–18	3	Kodsueb et al. (2006)
D. meiosporum	17–28 × 6–8.5	3–4	Liu et al. (2015)
D. micronesiacum	20–30 × 10–12	3–5	Goh et al. (1999)
D. musae	45–65 × 20–27	7	Photita et al. (2002)
D. nigroapice	28–41 × 15–20	4	Goh et al. (1999)
D. oblongum	30–50 × 12–30	3–6	Goh et al. (1999)
D. pandani	22–48 × 14–28	4–5	Whitton et al. (2012)
D. polystichum	26–34 × 23–34	(5–)7–9	Goh et al. (1999)
D. rhopalostylidis	28–48 × 20–29	5–6	McKenzie (2010)
D. schizostachyfolium	15–17 × 11–12	4	Goh et al. (1999)
D. sinense	50–130 × 20–35	4–7	Zhang (2009)
D. stellatum	50–175 × 27.5–52	5–7	Crous et al (2011)
D. strelitziae	30–55 × 20–23	4–6	Crous et al (2009)
D. subramanianii	33–42 × 16–21	3–7	Goh et al. (1999)
D. taishanense	27–43 × 15–30	(3–)5(–7)	Zhao & Zhang (2003)
D. tetraploides	52–72 × 18–26.5	5	Cai et al. (2003a)
D. tetraseriale	24–40 × 14–20	4	Goh et al. (1999)

Table 1, concluded on p. 1132

Table 1, concluded

Species	Conidial size (µm)	No. of rows	Reference
D. tetrasporum	23.5–40 × 16–21.5	4	Cai & Hyde (2007)
D. thailandicum	15–35 × 14.5–21	5	Liu et al. (2015)
D. tnlakhanpalii	80–120 × 25–36	(4–)6(–8)	Manoharachary et al. (2007)
D. toruloides	38–56 × 25–32	6–8	Goh et al. (1999)
D. triramosum	40–60 × 10–13.5	(2–)3	Arambarri et al. (2001)
D. triseriale	26–32 × 16–18	3	Goh et al. (1999)
D. yunnanense	25–45 × 22–38	(5–)6(–7)	Cai et al. (2003b)
D. zeylanicum	26–40×13–25	5	Goh et al. (1999)
D. zhejiangense	25–35 × 17–24	(4–)5	Wongsawas et al. (2009)

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Synchaetomella aquatica sp. nov. from submerged leaves from Brazil

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Abstract — *Synchaetomella aquatica,* a new microfungus collected on the submerged decaying leaves of *Calophyllum brasiliense*, is described and illustrated. It is distinguished by conidiogenous cells that are synnematal, determinate conidiomata, monophialidic, discrete, and terminal and solitary falcate unicellular hyaline conidia.

Key words — asexual fungi, systematics, freshwater fungi

Introduction

During the studies of saprobic conidial fungi colonizing submerged leaves of *Calophyllum brasiliense* in the Brazilian semiarid Caatinga biome, an interesting fungus belonging to the genus *Synchaetomella* was collected. Critical morphological observation of the specimen revealed it to be an undescribed species, which we describe here as *S. aquatica*.

Materials & methods

During several expeditions between November 2013 to May 2014 through "Serra da Tromba" in the semiarid region northeastern Brazil, samples of submerged leaves of *Calophyllum brasiliense* were stored in litter bags, taken to the laboratory, and processed according to Castañeda Ruiz (2005). Mounts were prepared in PVL (polyvinyl alcohol and lactic acid) and measurements were made at a magnification of ×1000. Micrographs were obtained with an Olympus microscope (model BX51) with bright field and Nomarski interference optics. The type specimen of the new species is deposited in the Herbarium of "Universidade Estadual de Feira de Santana" (HUEFS).

Taxonomy

Synchaetomella aquatica Fiuza, Gusmão & R.F. Castañeda, **sp. nov.** Figs 1, 2

MycoBank MB812900

Differs from all other *Synchaetomella* spp. by its exclusively terminal conidiogenous cells.

Type: Brazil, Bahia, Piatã, Chapada Diamantina, Serra da Tromba, 13°07′S 41°50′W, on decaying submerged leaves of *Calophyllum brasiliense* Cambess. (*Calophyllaceae*), 11.XI.2013, coll. P.O. Fiuza (**Holotype**, HUEFS 209015).

Etymology: Latin, *aquatica*, referred to its growing in water.

On the natural substratum mycelium partly immersed and partly superficial. Hyphae septate, branched, smooth, pale brown, 2–3 µm diam. Conidiomata synnematal, determinate, scattered, erect, cylindrical, dark brown, 60–150 µm tall, 25–30 µm wide. Stipe composed of parallel hyphal filaments (conidiophores), multi-septate, straight, cylindrical, closely packed at the base and slightly loosely and branched towards the apex, brown below, brown or pale brown above, smooth, 1.5–3 µm diam. Conidiogenous cells monophialidic, discrete, terminal, determinate, brown to pale brown, 15–45 × 1.5–2.5 µm with a narrow, slightly obscure, sub-cupulate collarette with a distinct, very narrow periclinal channel, 1–2.5 µm deep. Conidia solitary, acrogenous, falcate, unicellular, 8–18 × 1.5–2 µm, smooth, hyaline, accumulating in a white mucilaginous mass.

Note: Decock et al. (2005) introduced the synnematous genus *Synchaetomella* Decock & Seifert, typified by *S. lunatospora* Decock et al. and characterized by phialidic, terminal and intercalary conidiogenous cells and falcate, 0–1-septate, hyaline conidia. The second species, *S. acerina* (Seifert & Louis-Seize 2012), also has intercalary and terminal conidiogenous cells, but the conidia are unicellular, allantoid to ellipsoidal. *Synchaetomella aquatica* is clearly differentiated by the terminal subulate conidiogenous cells.

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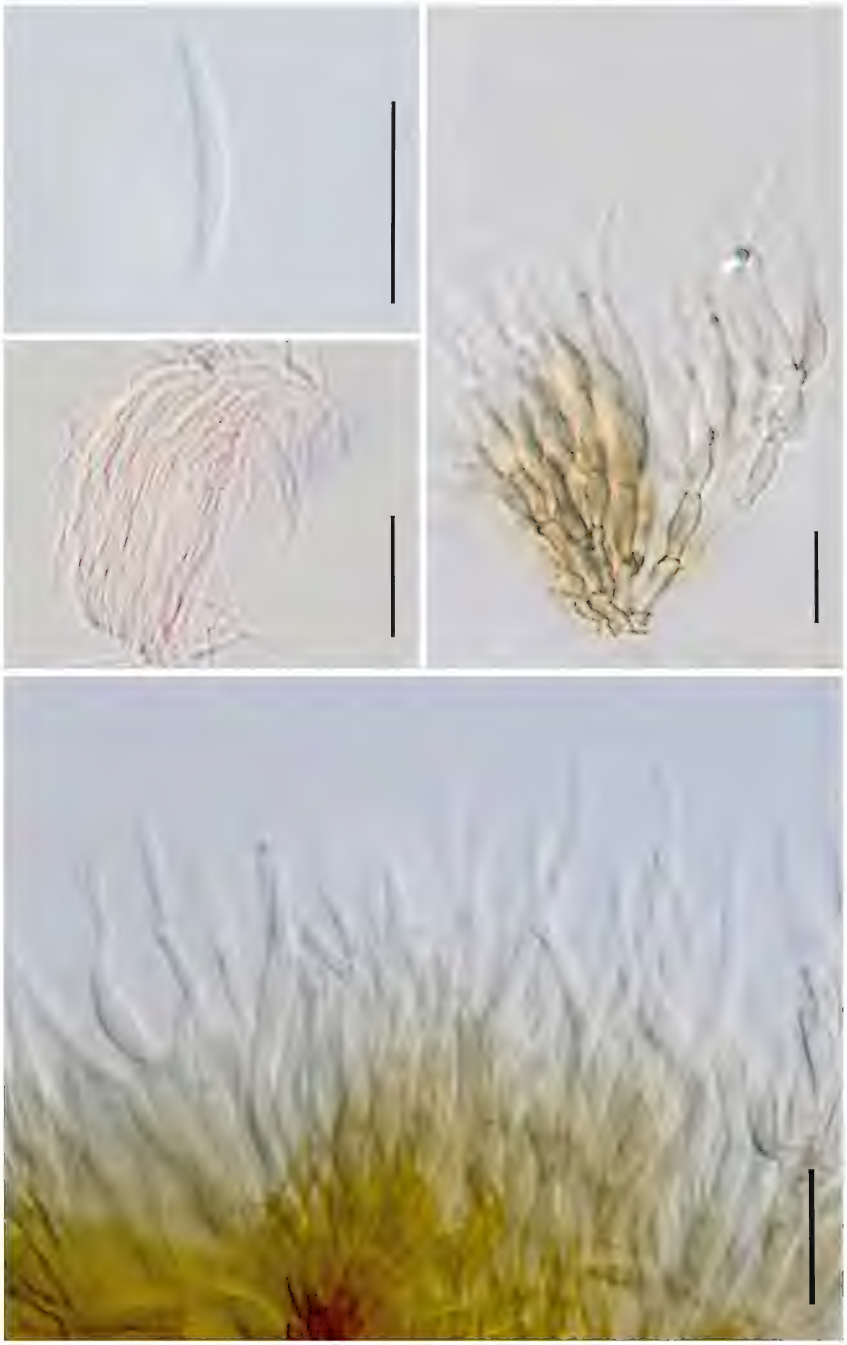
Fig. 1. *Synchaetomella aquatica* (ex holotype HUEFS 209015). A, B. conidia; C, D. conidiogenous cells and conidia. Scale bars: A, C, D = 10 µm, B = 20 µm.



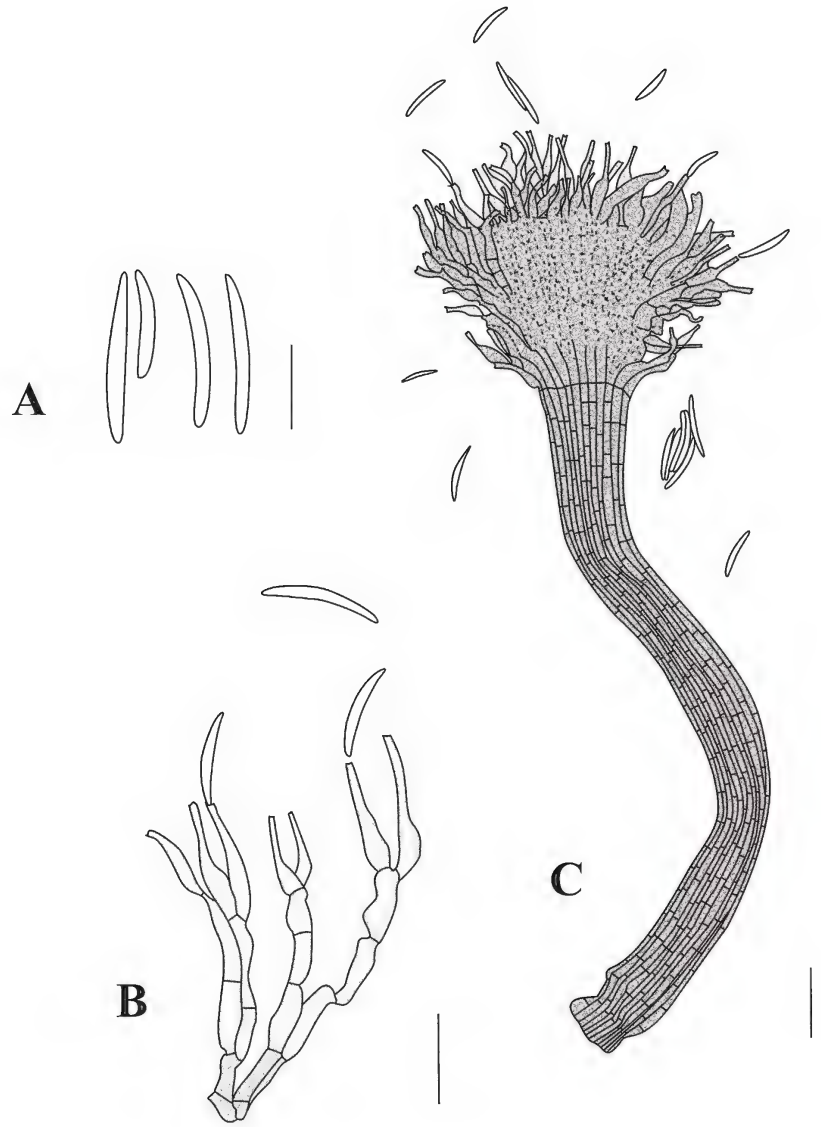


Fig 2. *Synchaetomella aquatica* (ex holotype HUEFS 209015).
A. conidia; B. conidiogenous cells and conidia; C. synnema.
Scale bars: A, B = 10 µm; C = 25 µm.

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Tuber petrophilum, a new truffle species from Serbia

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Abstract — *Tuber petrophilum* sp. nov., within the *Tuber melanosporum* lineage, is described from Mount Tara (western Serbia) based on morphological and ITS molecular data. It is recognizable by its minute ascomata that produce ovoid to ellipsoid to subfusiform spores bearing aculeate ornamentation. Among black truffles, the new species is distinguished by its irregularly roundish to subglobose ascomata not exceeding 1.6 cm in diameter, with a basal depression or cavity and peridium surface which appears as a thin semi-transparent layer while fresh. The species forms a monophyletic well-supported clade in Maximum Likelihood ITS phylogeny, closely related to *Tuber brumale* aggr. The distinctive feature of the new species lies in its specific and unique microhabitat, limited to humus-rich substrata accumulated as soil pockets in limestone rocks, commonly 20–100 cm above the continuous forest soil terraces. The species description is supplemented with macro- and micro-photographs, and a key to the species of the *T. melanosporum* lineage.

Key words — biodiversity, ecology of truffles, hypogeous fungi, *Tuberaceae*

Introduction

On several occasions between 2004 and 2014, the first author with associates explored hypogeous fungi in the Tara National Park on Mount Tara, which is located in western Serbia on the edge of northeastern belt of the Dinaric Alps. This area is a significant refugium for many endemic and relict plant species (Stevanović et al. 1995; Cvjetićanin & Novaković 2007, 2010). Apart from several hypogeous species found in quite usual habitats that were already known from other areas of Serbia, a few black truffles were discovered in an unusual and atypical habitat: cavities, crevices, and recesses in limestone rocks.

These specimens were readily distinguishable by a unique aroma, ascomatal size, and structure that did not correspond to any previously described species.

This paper aims to (1) describe a new species based on both morphological and molecular data, (2) ascertain the ecology of the species, and (3) provide an identification key and a phylogram to the *Tuber melanosporum* lineage.

Materials & methods

Sampling sites and collection treatment. — Our morphological examination and description of *Tuber petrophilum* is based on eleven collections comprising 26 ascomata from five localities in western Serbia on Mount Tara during September and October, 2004–14. All sites are situated within a narrow belt, a few hundred meters wide, along the local Mitrovac–Kaluđerske Bare road. The distance between the two furthest sites is approx. 6.7 km. Ascomata were discovered by a trained dog. Vouchers are conserved in the fungal reference collection of the Natural History Museum, Belgrade, Serbia (BEO), the fungal reference collection of Ljubljana University, Slovenia (LJU), and the private herbarium of M. Marković, Bela Crkva, Serbia. Ascomata were preserved by drying or by ethanol-glycerin fixative (70% ethanol to which 15% of glycerol was added). Sampling was done in representative forest communities in a limestone-dominated area where hypogeous fungal biodiversity was completely unknown. Climate data are available for the nearby meteorological station at Mitrovac (1082 m asl). Over the last 25 years, the yearly average temperature at the station was 5 °C and mean annual precipitation was 980 mm.

Description of hues uses the Pantone colour standard (Eiseman & Lawrence 1990). The letter "P" followed by the number in parentheses represents the Pantone code. Microscopic characters of *Tuber petrophilum* were examined in both fresh and dry material with a Leica Leitz DMRB microscope equipped with a Leica DFC320 digital microscope camera and Leica DMLS microscope equipped with Leica DFC295 digital microscope camera. Spores were measured in 5% KOH in a bright field microscopy using Zeiss Axio Imager.Z2 microscope (100–1000× magnification) with the automated measuring function in ZEN pro 2011 software. Spores considered to be not full-size, anomalous, or aberrant were excluded. The length and width of full-sized spores were measured in side view. Ornamentation was excluded from spore measurements and measured separately. The spore size quotient Q represents the length : width ratio range determined from all measured ascospores. The mean quotient Q_m was calculated as arithmetic average of the Q values from all ascospore measured. Twenty spores were measured for each collection.

As truffles are known for their specific soil requirements, several basic soil characteristics at the three most productive localities (Dolak [the holotype site], Tisovo brdo, and Rustine), were analyzed. About 0.5 L of soil was taken around the ascomata and analyzed for humus, phosphorus (P_2O_5) and potassium (K_2O) content, and pH. Standard laboratory analyses of collected soil samples included the Kotzman method for humus content, measurement of pH by placing a glass electrode in 1M KCL solution, Egner-Rhim Al flame photometry method for potassium, Egner-Rhim Al colorimetry method for phosphorus, and determination of calcium carbonate by

Scheibler volumetric method (Tan 1998). Analyses were performed at the Institute for Soil Science (Belgrade, Serbia).

DNA extraction, PCR amplification and sequencing. — DNA was extracted from four dried glebal samples from a mature and sufficiently large *Tuber petrophilum* ascoma using a Qiagen DNeasy Plant Mini Kit. DNA amplification using general ITS primers ITS1f combined with ITS4 (White et al. 1990, Gardes & Bruns 1993) followed the modified protocol in Grebenc et al. (2009). Prior to sequencing, products were cleaned from the gel using the Promega Wizard SV Genomic DNA Purification System. Both strands were sequenced separately at Macrogen Korea (Seoul, Korea). All samples were sequenced in both directions using the same primers as in the amplification. Sequencher 5.1 was used to assemble the consensus sequence from the two strands of the ITS nrDNA of each isolate. Complete ITS sequences were deposited in GenBank under accession numbers HG810883–810886.

Phylogenetic analyses. — All obtained consensus sequences were aligned with a selection of *Tuber* sequences from the International Nucleotide Sequence Databases using the stand-alone freeware version of the MAFFT programme (http://align.bmr.kyushu-u.ac.jp/mafft/software/) with the E-INS-i aligning strategy (Katoh et al. 2005). *Reddellomyces* sp. (GenBank: HM485336 and HM485337) was used as outgroup as a close *Tuber* relative from the southern hemisphere with a basal position according to Bonito et al. (2013). The MEGA 5.10 package was used to construct Maximum Likelihood (ML) phylogenetic reconstruction based on a Tamura-3-parameter substitution model with Gamma distributed rates among sites including invariant sites. In the dataset gaps / missing data were partially deleted and 95% site coverage cutoff and a strong branch swap filter were applied. 2000 bootstrap replicates were run. The Maximum Parsimony (MP) reconstruction and branch support was based on a MEGA 5.10 incorporated Subtree-Pruning-Regrafting MP search method with 2000 replicates, gaps / missing data were partially deleted, and a 95% site coverage cutoff was applied. MEGA 5.10 package was used also to annotate the final tree and collapse branches at species level.

Taxonomy

Tuber petrophilum Milenković, P. Jovan., Grebenc, Ivančević & Marković, **sp. nov.** Plate 1

MycoBank MB812245

Differs from all other species of the *Tuber melanosporum* lineage by its considerably smaller mature ascomata, its reddish-black peridium with a thin semi-transparent layer when fresh, its spores with shorter ornamentation, and its distinctive habit in accumulated soil pockets in rocks.

Type: Serbia, Bajina Bašta, Tara National Park, Mitrovac, Dolak, 43.924810°N 19.462610°E, 1015 m asl, under *Fagus sylvatica* & *Abies alba*, in humus-rich substrate accumulated as soil pockets in limestone rocks, commonly 20–100 cm above the continuous forest soil terraces, 5 ascomata, 28 Sep 2004, leg. M. Milenković (**Holotype,** BEO 20600).

Etymology: in reference to the specific habitat of the species – *petrophilum* (Gr. πέτρα = stone, rock; φίλος = who love).

Ascomata strictly hypogeous, solitary or in small groups, rather small, ranging from 8–16 mm in diam., nearly globose to irregularly subglobose, with basal depression or cavity, firm.

Peridium reddish-black (P 19-1015), when fresh, surface appears as a thin semi-transparent layer, turning to non-transparent and completely black when dried, pseudoparenchymatous, total thickness 150–450 µm (average 320 µm), with melanised outer layer, 20–200 µm, without hyphal outgrowth. Warts angular, flattened and depressed at the apex, 0.6–1.2 mm diam., 0.3–0.7 mm high.

Gleba solid, whitish at first, becoming brown (P 18-1137) and finally dark brown (coffee brown; P 18-1154), at maturity with wide, infrequently (1–3×) branched white veins, which radiate from the base and terminate close to the peridium. Asci from fresh ascomata subglobose to irregular with well-developed stalk, (1–)2–4(–6, extremely rarely)-spored, 40–50 × 55–80 µm, spores when inside asci randomly disposed and loosely packed. Ascospores 22.7–42.5 × 13.1–26 µm, symmetrical to asymmetrical, ovoid to ellipsoid to subfusiform, Q = 1.37–2.29, Q_m = 1.68 (SD = 0.16); at first hyaline to bright brownish (P 15-1225), later brown (P 16-1422/17-1430) to dark brown (P 17-1422) (resembling *T. melanosporum* Vittad.); ornamented with straight or sometimes slightly curved, concolorous, dense, separate spines, 2–4(–4.5) µm high and 0.4–0.6 µm diam., 1–2.5 µm diam. at the base.

Odour of mature ascomata intensive and specific, pleasant, resembling a mixture of the aromas of *Tuber borchii* Vittad. and *Tuber brumale* Vittad.

Flavor not observed.

Additional specimens examined: **SERBIA, Bajina Bašta, Tara National Park**, Mitrovac, Dolak, 43.924810°N 19.462610°E, 1015 m asl, under *Fagus sylvatica* & *Abies alba*, 3 ascomata, 12 Oct. 2014, leg. M. Milenković & B. Ivančević (BEO 20606, 20607, 20608); Mitrovac, Tisovo brdo-Lokvica, 43.932980°N 19.448070°E, 1084 m asl, under *F. sylvatica*, *A. alba*, and *Picea abies*, 2 ascomata, 29 Sep. 2004, leg. M. Milenković (BEO 20601); 4 ascomata, 8 Oct. 2010, leg. M. Milenković (LJU GIS TUBPET/xx1010A, B, C, D; GenBank HG810883–810886); Mitrovac, Tisovo brdo, c. 43.927220°N 19.443649°E, 1075 m asl, under *F. sylvatica*, *A. alba* & *P. abies*, 1 ascoma, 29 Sep. 2004, leg. M. Milenković (herb. Marković); Mitrovac, Children's resort, 43.922180°N 19.421160°E, 1062 m, under *F. sylvatica* & *P. abies*, 3 ascomata, 5 Oct. 2006, leg. M. Milenković (herb. Marković); 1 ascoma, 11 Oct. 2014, leg. M. Milenković & B. Ivančević (BEO 20605); Šljivovica, Kovači, north slopes, 43.917050°N 19.491500°E, 1111 m, under *F. sylvatica* & *A. alba*, 2 ascomata, 9 Oct. 2010, leg. M. Milenković (herb. Marković); 2 ascomata, 11 Oct. 2014, leg. M. Milenković & B. Ivančević (BEO 20603, 20604); Šljivovica, Rustine, 43.912210°N 19.502850°E, 1029 m, under *F. sylvatica*, *A. alba* & *P. abies*, 2 ascomata, 18 Oct. 2010, leg. M. Milenković (herb. Marković); 1 ascoma, 11 Oct. 2014, leg. M. Milenković & B. Ivančević (BEO 20602).

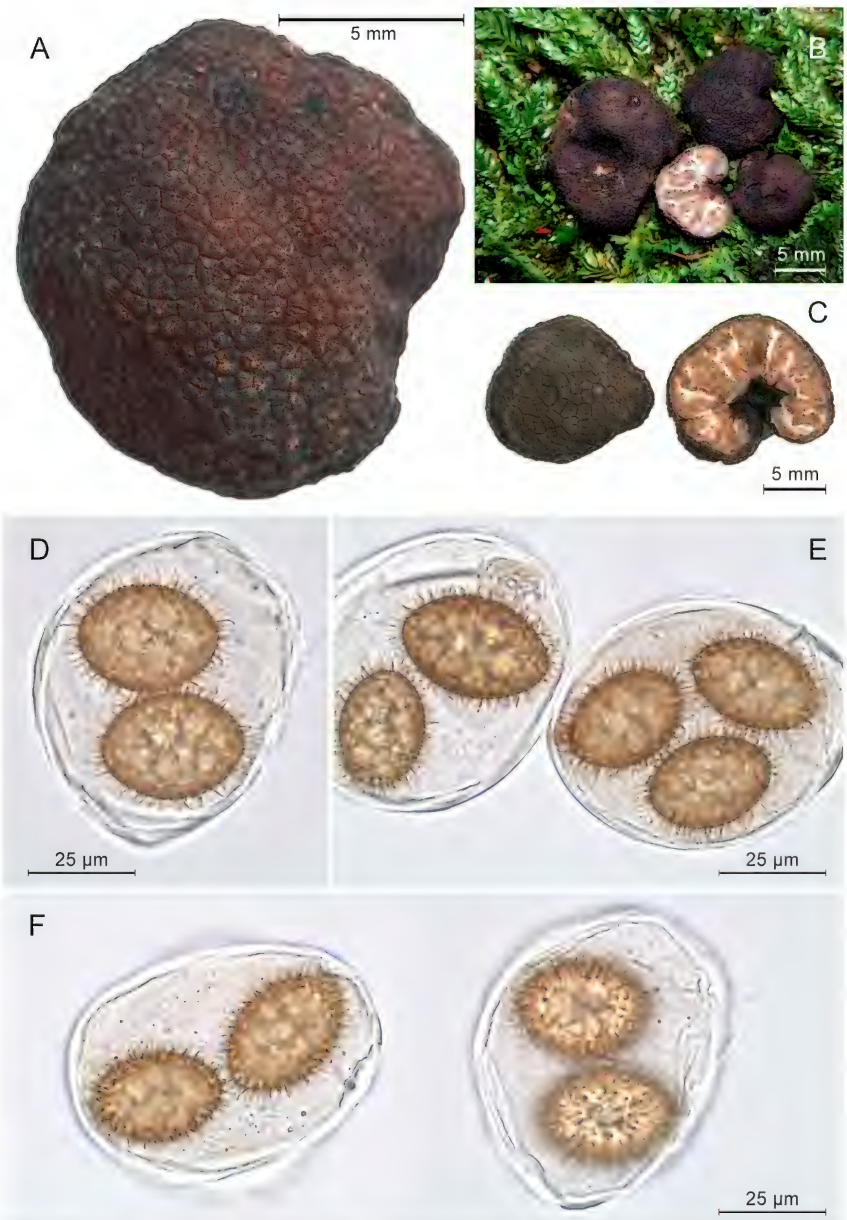

Plate 1. *Tuber petrophilum*: A. Fresh ascoma (BEO 20604). B, C. Fresh ascomata and cross section (B: BEO 20600: C: BEO 20608). D–F. Asci with spores from fresh ascomata (D, F: BEO 20608; E: BEO 20600).

Habitat — All ascomata were found in humus-rich substrata accumulated as soil pockets in limestone rocks, 5–12 cm below the surface of the substratum and commonly 20–100 cm above the continuous forest soil terraces, formed on limestone mountain slopes of Mount Tara. The forest type is the continental montane forest dominated with *Fagus sylvatica* L., *Abies alba* Mill., and *Picea abies* (L.) H. Karst. forming an association *Piceo-Abietetum* Čolić 1965 (Tomić 2006, Tomić & Rakonjac 2011) at altitudes from 1000–1100 m asl.

Phenology — September to October.

Distribution — Known only from the type locality and its environs in Serbia.

Discussion

Tuber P. Micheli ex F.H. Wigg. is among the best studied hypogeous genera in Europe (Hall et al. 2003). Until recently, about 32 different *Tuber* species had been described in Europe (Ceruti et al. 2003) and at least 180 species estimated worldwide (Bonito et al. 2010). *Tuber petrophilum*, unlike other hypogeous fungi, inhabits accumulated soil pockets in rocks, partly isolated from the surrounding continuous soil layer.

A combination of macro- and microscopic characters together with its unique ecology separate *Tuber petrophilum* from all other taxa in the *T. melanosporum* lineage (Riousset et al. 2001, Bonito et al. 2013), and its mature ascomata are considerably smaller than any other closely related species. Molecular data support its position in the *T. melanosporum* lineage and significantly support its separation from close relatives *T. brumale* aggr. and *T. pseudoexcavatum* Y. Wang et al. Spore size range and ornamentation size are key microscopic characters that distinguish it from species in the *T. melanosporum* lineage.

Tuber petrophilum forms a separate, well supported clade in the nrITS1-5.8S-ITS2-based phylogeny (Plate 2). The closest ITS-based relatives are the European *Tuber brumale* aggr., *T. pseudoexcavatum* (Asia), *T. regimontanum* Guevara et al. (Central America), and *T. melanosporum* (Europe). We have observed a persistent presence of a c. 300-bp intron in the ITS1 region of *T. petrophilum* and *T. brumale* aggr. but not in other species in the *T. melanosporum* lineage. Excluding the intron, *T. petrophilum* shared 82–85% sequence similarity with other lineages within the *T. brumale* aggr. and ≤82% similarity with other species in the *T. melanosporum* lineage.

The ecology of *T. petrophilum* is strikingly distinct within the *T. melanosporum* lineage. While other species are found primarily in Mediterranean or Continental climates with both hot summer and moderate winter periods (Montecchi & Sarasini 2000, Riousset et al. 2001), *T. petrophilum* was discovered in a colder, montane climate at higher elevations and on different soil types. In

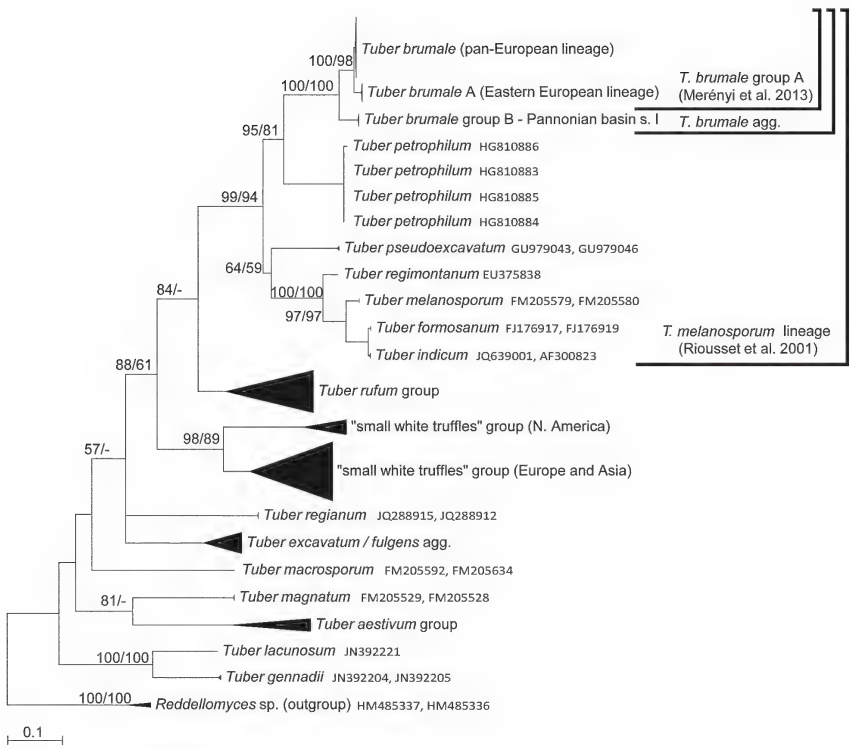

PLATE 2. nr ITS1-5.8S-ITS2 phylogenetic reconstruction of the genus *Tuber* with a particular focus on the *T. melanosporum* lineage, with *T. petrophilum* as a neighbouring clade to *T. brumale* aggr. and *T. pseudoexcavatum*. The phylogenetic reconstruction is based on 140 sequences selected from well characterised collections deposited in the International Nucleotide Sequence Databases. Values for ML and MP branch supports are given for nodes with ≥50 bootstrap support.

particular, pH and humus contents deviate from requirements and ecological limits of other European species in the *T. melanosporum* lineage (Callot et al. 1999, Riousset et al. 2001). Site conditions for *T. petrophilum* are unique: its small size permits it to fruit well in small, humus-rich, and slightly acidic accumulations of soil in rock pockets that are physically separated from nearby soils. All specimens collected at different sites in the course of ten years were found in such pockets (cavities) or crevices in larger and smaller limestone rocks jutting from the soil, commonly 20–100 cm above the surrounding soil (PLATE 3). No ascomata were collected in the nearby continuous soil

Plate 3. *Tuber petrophilum*: Typical microhabitat inside humus rich soil pocket in limestone rocks. A–C. Red arrows indicate the exact location where ascomata were unearthed. D. The substrate inside the hole is visible after removing the litter. E, F. The cavity containing ascomata.

layer, despite careful searching with the aid of a trained dog. In this unique microhabitat, humus mixed with eroded stone particles form a black, loose and well-aerated substratum. Chemical analysis of the *T. petrophilum* substratum confirmed high humus content (11.8–13.6%), with phosphorus measured at 5.15–11.70 mg/100 g and potassium at 24.35–34.06 mg/100 g. The pH of the substratum ranged from 6.3 to 7.0. This indicates that *T. petrophilum* is

a highly demanding species with regard to the habitat requirements, which include presence of adequate rock cavities with a humus-rich substratum and simultaneously accessible to mycorrhizal roots of an appropriate tree species, which we assume to be *Fagus sylvatica*. Fructification appears limited to years with sufficient precipitation throughout the year, as mature ascomata were found in September and October (until snowfall) only in years without prolonged drought. The sporadic and irregular fruiting of only a small number of ascomata is probably caused by the combination of the karstic habitat and climatic conditions.

Tuber petrophilum has been found in only a few localities within a very small area, suggesting that it is a very rare species. Although *Fagus sylvatica* along with *Abies alba* stands are not rare in Serbia and throughout the Dinarides, here in combination with karst terrain, rocks, and soil a unique habitat was formed. Sites are near the road, in a National Park zone with the lowest regime of protection where forest exploitation is allowed, and felling was launched in the immediate vicinity of where the new species was found. On that basis, *T. petrophilum* is clearly threatened given its small known population and endangered habitat. Research on distribution, population trends, and ecological requirements of the species is needed, as well as protection measures and monitoring for the known population and sites.

The small size of the *T. petrophilum* ascomata and its relatively scarce fruiting is unlikely to establish the species as commercially or gastronomically significant, despite the high quality of its aroma.

Key to the species of *Tuber melanosporum* lineage

The *T. melanosporum* lineage differs from other lineages in the genus *Tuber* by black colour of the mature ascomata with spores bearing more or less pronounced warts or aculei.

1. Mature spores light brown to hazel brown, spines 3–6 µm long, thin; gleba with few or infrequent branching veins. 2
   Mature spores hazel brown to dark brown (when mature), spines 1.5–8 µm long, variably thick; gleba with few or many thin veins. 4
2. Spores 33–55(–62) × 23–31 µm, Q = 1.3–2.0, spines 2–5 µm long, thin; 1–4 spores per ascus; gleba solid, dark brown; described from Central America (Guevara et al. 2008) . . . . . *T. regimontanum*
   Spores generally smaller, 1–6 spores per ascus, Q not exceeding 1.8 3
3. Spores 25–31 × 16.5–18.5 µm, pan-European distribution, ITS-supported clade (Merényi et al. 2014) . . . . . *T. brumale* Group A
   Spores 25–34 × 15.5–19.5 µm, Pannonian basin (Europe) distribution, ITS-supported clade (Merényi et al. 2014) . . . . . *T. brumale* Group B

4. Spores dark brown, opaque; ascomata prominent, roundish to irregular, lobed, never with basal depression or cavity 5

   Spores brown to dark brown; ascomata with cavity or basal depression emerging from the basal point 6

5. Spores (20–)25–55 × 14–35 µm, spines 2–4(–6) µm long, thin, without wide base; 1–6 spores per ascus, Q = 1.4–2.1; gleba blackish brown with purple tinges, firm, with well-defined veins, reddening; aroma pleasant and characteristic, Mediterranean Europe (introduced worldwide) ..... *T. melanosporum*

   Spores 15–35(–40) × 10–30(–35) µm, spines robust, curved, 3–5 µm long, with wide base and forming incomplete meshes of a reticulum; 1–6 spores per ascus; gleba blackish brown, compact, veins well defined, infrequent, thin; described from Asia. [Species an aggregate of several phylogenetic species (Chen et al. 2011)] ..... *T. indicum* Cooke & Massee

6. Spores 12.5–25 × 10–18 µm, spines 3 µm long, truncate; gleba dark brown to black, with white to yellowish brown or purplish veins; described from *Fabaceae* and *Lauraceae* forests in southeast Asia (Huang et al. 2009) ..... *T. formosanum* H.T. Hu & Y. Wang

   Spores >23 µm long, brown to dark brown; ascomata from native forests in Europe or (when from Asia) then ascomata deeply excavated; aroma intense, pleasant 7

7. Spores 23–42.5 × 13–26 µm, spines straight or slightly curved, 2–4(–4.5) µm long; (1–)–4(–6) spores per ascus; gleba dark brown with wide, 1–3× branched white veins that radiate from the base and end close to peridium; aroma pleasant resembling mixture of *T. borchii* and *T. brumale;* described from the eastern edge of Dinaric mountains (W. Serbia) ..... *T. petrophilum*

   Spores (23–)24–28(–35) × 16–19(–22) µm, spines prominent, 5–8 µm long; 1–8 spores per ascus; gleba grey-brown with white sinuous veins; aroma intense; Asia, but introduced worldwide ..... *T. pseudoexcavatum*

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Pouzarella alissae, a new species from northwestern California, United States

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Abstract —*Pouzarella alissae* sp. nov. is known only from north coastal California. It is characterized by the evanescent silvery-white layer of fibrils and squamules initially covering the pileus and stipe surface, the dark greyish brown to dark brown innately fibrillose pileus and stipe, a pileipellis of entangled hyphae with cylindro-clavate terminal cells, a suprapellis with cytoplasmic pigments, a subpellis with encrusted pigments, heterogeneous lamellar edges, lageniform to rostrate-ventricose leptocystidia, cheilocystidia and pleurocystidia, abundant aborted basidia, and basidiospores averaging <11 µm in length.

Key words —*Basidiomycota*, *Entolomataceae*, phylogeny, taxonomy

Introduction

The name *Pouzarella* is applied to fungi in the *Entolomataceae* that possess stipitate–pileate basidiomata with the following features: basidiome typically mycenoid with a strigose stipe base, pileipellis disc composed of erect to semi-erect multicellular hyphae, tramal hyphae with encrusted pigments, nodulose heterodiametric 5–9-angled basidiospores, and the absence of clamp connections. Phylogenetically, these fungi form a well-supported clade (He et al. 2013), and *Pouzarella* Mazzer is applied as a generic concept (Mazzer 1976; Karstedt et al. 2007; Baroni et al. 2008, 2012; Horak 2008) or is treated as *Entoloma* subg. *Pouzarella* (Mazzer) Noordel. (Manimohan et al. 2006, Noordeloos 2004, He et al. 2013). We treat *Pouzarella* as a genus because all species form a monophyletic group with distinctive morphological characters.

Although *Pouzarella* species are distributed worldwide, they are encountered most frequently in mesic areas that receive >20 in. annual rainfall (Mazzer 1976). In North America, *Pouzarella* is common in the eastern United States

and Canada, but is rare in the west where it is restricted to a narrow coastal region extending from southern California to southern British Columbia (Largent 1994, Mazzer 1976).

Of the fifty-nine *Pouzarella* species reported worldwide in Index Fungorum, four have been described from western North America: *P. fernandae* (Romagn.) Largent and *P. fulvostrigosa* (Berk. & Broome) Mazzer from Washington, *P. araneosa* (Quél.) Mazzer from California, and *P. versatilis* (Gillet) Mazzer from California, Oregon, and Washington (Largent 1994, Mazzer 1976). In this report, we describe a third and new species from California, *P. alissae*.

Materials & methods

Macromorphological and micromorphological features

Techniques and equipment for collecting and describing basidiomata in the field and GPS coordinates have been described in Largent et al. (2011a,b), while techniques for color descriptions using Kornerup & Wanscher (1978) and factors determined from mathematical analyses in the descriptions are covered in Largent et al. (2013a,b). Dried specimens were examined microscopically with a research-grade trinocular Nikon Eclipse Ci-L compound microscope. Microphotographs were made using a Lumenera Infinity 2-5 CCD digital camera, and all microscopic measurements were obtained using Infinity Analyze software 6.5.0.

DNA sequences and phylogenetic analyses

Sequences of *P. alissae* were obtained from the mitochondrial small subunit ribosomal RNA (mtSSU), the nuclear large subunit ribosomal RNA (LSU), and second largest subunit of RNA polymerase II (RPB2) following protocols described in Largent et al. (2011b). Sequences were edited using Sequencher 4.2.2 (Gene Codes, USA) and aligned using MAFFT v. 7 (Katoh & Standley 2013). Thirty-eight sequences were generated for this phylogenetic analysis along with sequences obtained from GenBank including those for the majority of *Pouzarella* species and from species from other representative clades as defined in Baroni & Matheny (2011) (Table 1).

Table 1. Collections used in the phylogenetic analyses.
New sequences generated for this study are shown in bold.
Square brackets enclose GenBank names differing from those in Fig. 1.

Taxa	Collection identifier	GenBank accession numbers		
		mtSSU	LSU	RPB2
Alboleptonia angustospora [*Alboleptonia* aff. *sericella*]	MCA1978	GU384583	GU384609	GU384632
Alboleptonia sericella [*Entoloma sericellum*]	10	GQ289330	GQ289190	GQ289261
Catathelasma imperiale	11CA001A	**KR869913**	**KR869941**	KC816816
Claudopus minutoincanus	DLL9871	HQ731511	HQ731514	HQ731517
Claudopus parasiticus	10617 TJB	**KR233902**	**KR233872**	**KR233924**
Claudopus viscosus	DLL9788	HQ31513	HQ31516	HQ31518
Clitocella fallax [*Rhodocybe fallax*]	25668OKM	**KR869909**	AF261283	KC816937

Clitopilopsis hirneola	REH8490	GU384587	GU384611	KC816904
Clitopilus apalus	26394WAT	**KR869907**	**KR869936**	KC816906
Entocybe haastii [*Entocybe* sp.]	DLL10087	JQ793645	JQ793652	JQ793659
Entocybe trachyospora [*Rhodocybe trachyospora*]	5856 TJB	GU384605	GU384629	GU384658
Entoloma abortivum	5772 TJB	**KR233897**	**KR233869**	**KR233926**
E. albidoquadratum	i4	GQ289291	GQ289151	GQ289223
E. bloxamii	219	GQ289294	GQ289154	GQ289226
E. caespitosum	GDGM 27564	JQ993070	JQ410327	JQ993078
E. changchunense	HMJAU 3886	JQ993061	JQ993095	—
E. crassicystidiatum	GDGM 28821	JQ993058	JQ291567	JQ993083
	GDGM 27357	JQ993056	JQ291569	JQ993085
E. discoloratum	DLL10217	JQ793646	JQ793653	JQ793660
E. fragilum	MCA2415	KJ021690	KJ021700	KJ021694
E. furfuraceum	GDGM 28818	JQ993062	JQ993094	JQ993084
E. indoviolaceum	i13	GQ289312	GQ289172	GQ289243
E. myrmecophilum	231	GQ289314	GQ289174	GQ289245
E. omiense	GDGM 27563	JQ993067	JQ410330	JQ993079
E. pallidocarpum	GDGM 28828	JQ993074	JQ410331	JQ993080
E. praegracile	GDGM 29251	JQ993072	JQ320129	JQ993077
E. procerum	70	GQ289323	GQ289183	GQ289254
E. prunuloides	40	GQ289324	GQ289184	GQ289255
E. readiae	102	GQ289326	GQ289186	GQ289257
E. rhodopolium	8	GQ289327	GQ289187	GQ289258
E. rugosiviscosum	DLL9676	JQ793647	JQ793654	JQ793661
E. sericatum	28	GQ289329	GQ289189	GQ289260
E. sinuatum	50	GQ289333	GQ289193	GQ289264
E. subaraneosum	KA12-1534	—	KJ523137	—
	GDGM 28823	—	JQ291568	—
E. aff. *subsinuatum* [*E. sinuatum*]	5349 TJB	**KR233896**	**KR233868**	AY691891
E. tenuissimum	GDGM 28813	JQ993059	JQ993097	JQ993086
	GDGM 28814	JQ993060	JQ993096	JQ993087
E. undatum	18	GQ289342	GQ289202	GQ289270
E. valdeumbonatum	189	GQ289343	GQ289203	GQ289271
E. violaceotinctum	DLL10088	JQ793643	JQ793650	JQ793657
E. violaceovillosum	i2	GQ289345	GQ289205	GQ289273
E. yunnanense	GDGM 28815	JQ993057	JQ320128	—
Inocephalus hypipamee	DLL10071	JQ624604	JQ624609	JQ624616
I. "*lactifluus*"	7962 TJB	—	AF261304	—
I. plicatus	DLL9691	JQ624605	JQ624610	JQ624617
I. virescens	DLL9772	**KR233889**	**KR233859**	**KR233918**
Lepista nuda	11CA041	**KJ021692**	**KJ021705**	KJ136110
Leptonia ambigua	DLL9872	JQ756399	JQ756414	JQ756429
L. boardinghousensis	DLL10086	JQ756398	JQ756413	JQ756428
L. omphalinoides	DLL9673	JQ756400	JQ756416	JQ756431
L. odorifera	6534 TJB	KR233899	KR233870	KR233927
L. poliopus	DLL10209a	JQ756402	JQ756418	JQ756433

L. serrulata [*Entoloma serrulatum*]	163	GQ289332	GQ289192	GQ289263
L. "subdecurrentiba" [*L.* sp.]	MCA1486	GU384589	GU384623	GU384635
L. tjallingiorum [*Entoloma tjallingiorum*]	243	GQ289337	GQ289197	GQ289267
L. trichomata	11CA006	**KR233873**	**KR233873**	**KR233903**
L. umbraphila	DLL9793	JQ756409	JQ756424	JQ756440
Mycena aff. *pura*	11CA007	**KR869914**	**KR869942**	KC816995
Nolanea cetrata	DLL9531	KF738927	KF738942	KF771346
N. cf. conferenda [*Entoloma conferendum*]	6	GQ289300	GQ289160	GQ289231
N. hebes [Entoloma hebes]	46	GQ289310	GQ289170	GQ289241
N. sericea	DLL9527	**KR233882**	**KR233851**	**KR233913**
N. strictior var. *isabellina* [*E. strictius* var. *isabellinus*]	7710 TJB	GU384594	GU384618	GU384641
Panellus stipticus	11CA052	**KR869915**	**KR869943**	KC816996
Paraeccilia sericeonitida [*Entoloma sericeonitidum*]	7144 TJB	EF421098	AF261315	EF421016
Pouzarella albostrigosa	DLL9641	HQ876557	HQ876535	HQ876513
	DLL9663	HQ876558	HQ876536	HQ876514
P. alissae	DLL10490	**KT074359**	**KT074357**	**KT074361**
P. araneosa [*Entoloma araneosum*]	14	GQ289293	GQ289153	GQ289225
P. debilis	DLL9784	HQ876550	HQ876528	HQ876506
P. farinosa	DLL9900	HQ876537	HQ876515	HQ876494
	DLL9934	HQ876538	HQ876516	HQ876495
P. fusca	DLL9623	HQ876549	HQ876527	HQ876505
P. lageniformis	DLL9895	HQ876545	HQ876523	HQ876501
P. lasia	DLL9662	HQ876551	HQ876529	HQ876507
	DLL9670	HQ876552	HQ876530	HQ876508
	DLL9729	HQ876553	HQ876531	HQ876509
	DLL9807	HQ876555	HQ876533	HQ876511
	DLL9811	HQ876556	HQ876534	HQ876512
P. farinosa	DLL9900	HQ876537	HQ876515	HQ876494
	DLL9934	HQ876538	HQ876516	HQ876495
P. nodospora	5716 TJB	**KT074358**	**KT074356**	**KT074360**
P. pamiae	DLL9794	HQ876539	HQ876517	HQ876496
	DLL9808	HQ876540	HQ876518	HQ876497
	DLL9834	HQ876541	HQ876519	HQ876498
P. parvula	DLL9901	HQ876546	HQ876524	—
P. pilocystidiata	DLL9848	HQ876542	HQ876520	HQ876499
P. setiformis	DLL9809	HQ876547	HQ876525	HQ876503
	DLL9810	HQ876548	HQ876526	HQ876504
Rhodocybe roseiavellanea	8130 TJB	**KR869901**	**KR869930**	KC816982
R. spongiosa	MCA2129	GU384604	GU384628	GU384657
Rhodophana nitellina	7861 TJB	**KR869900**	**KR869929**	KC816959
Tricholoma flavovirens	11CA038	KJ021691	KJ021704	KC816997
Trichopilus porphyrophaeus [*Entoloma porphyrophaeum*]	113	GQ289322	GQ289182	GQ289253

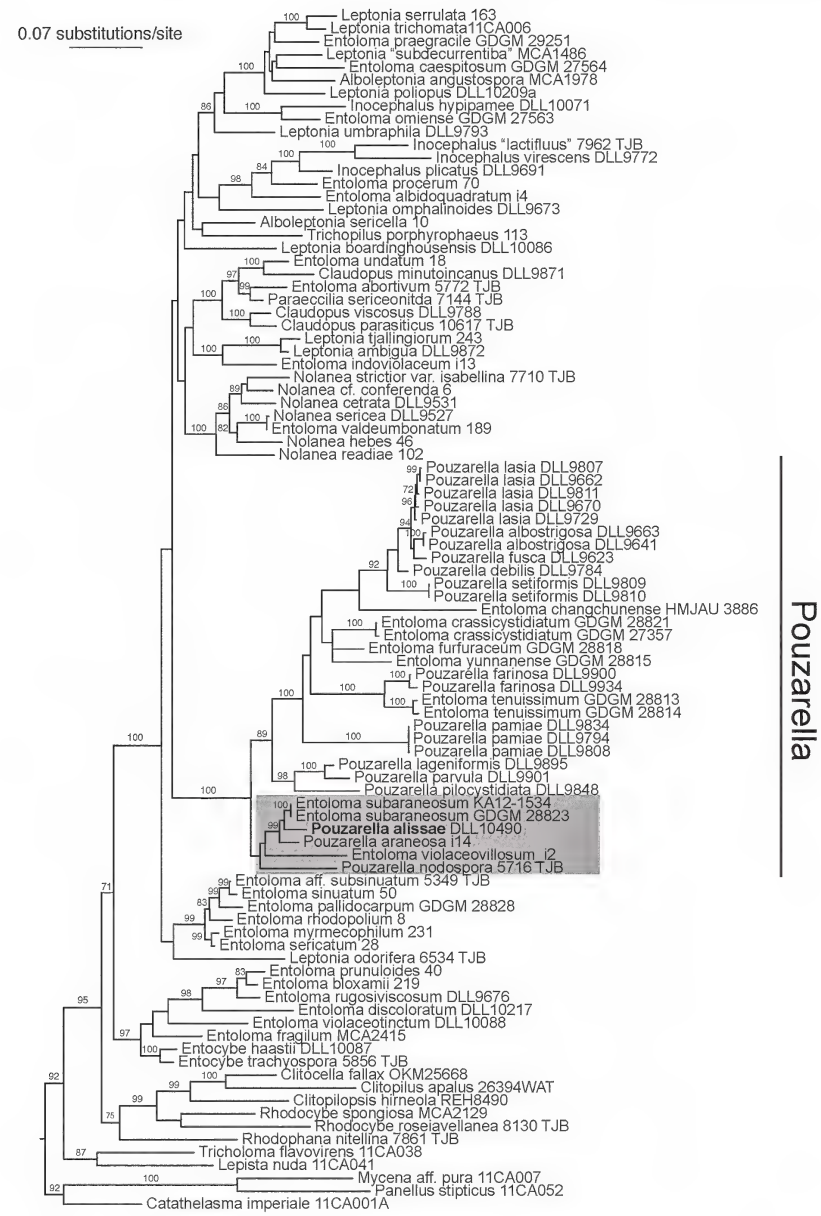

FIGURE 1. Maximum-likelihood topology, highlighting the sub-clade within *Pouzarella* containing the new species, *Pouzarella alissae*. Each sequence is labeled with the GenBank-listed isolate identifier. Branches with ≥70% support from 1000 rapid bootstraps are shown. *Catathelasma imperiale*, *Lepista nuda*, *Mycena* aff. *pura*, *Panellus stipticus*, and *Tricholoma flavovirens* were used as the outgroup.

After exclusion of the RPB2 introns and mtSSU hypervariable regions and manual optimization, alignment lengths were 592 bp (mtSSU), 1498 bp (LSU), and 1078 bp (RPB2). A Maximum Likelihood (ML) analysis based on a concatenated dataset with 1000 replicates and specifying a GTRGAMMA model using RAXML-HPC2 v. 8.1.11 (Stamatakis 2014) was carried out with a partitioned dataset for each RPB2 codon (three partitions) and separate partitions for mtSSU and LSU. One thousand bootstrap (BS) replicates were generated for assessment of branch supports.

Phylogeny

Monophyly of *Pouzarella* is well supported (BS = 100, Fig. 1), and *Pouzarella alissae* clusters with other representatives of *P.* sect. *Versatiles* (*P. araneosa* and *Entoloma subaraneosum*) with significant support (BS = 99, Fig. 1). These three species share similar features including a fibrillose pileus and stipe and thin-walled hymenial cystidia. Additionally, all three species have dark brown basidiomata, evanescent silvery-white coverings on stipe and pileus, appressed fibrillose pileipelli that become subglabrous, intracellular pigments in the pileus suprapellis, hyphae with encrusted pigments in the pileus subpellis and trama, and lageniform to rostrate-ventricose leptocystidioid cheilocystidia. They cluster within a larger clade with other members of *Pouzarella* sect. *Pouzarella* (*P. nodospora* (G.F. Atk.) Mazzer and *Entoloma violaceovillosum* Manim. & Noordel.), but without support (Fig. 1). Whether this lack of support for the morphologically based infrageneric classification is due to a lack of adequate taxon sampling or gene resolution will require further research.

Taxonomy

Pouzarella alissae Largent & Bergemann, **sp. nov.** Plates 1, 2

MycoBank MB 812300

Differs from *Pouzarella araneosa* by its dark brown pileus, shorter basidiospores, shorter cheilocystidia, and the presence of pleurocystidia.

Type — United States, California, Humboldt County, Prairie Creek Redwoods State Park, Intersection of Big Tree Trail and Prairie Creek Highway, in duff beneath *Acer circinatum* and *Polystichum munitum*, within 20 m of 41°22′33.15″N 124°00′51.66″W, 62 m elev., 22 November 2014, DL Largent 10490 (**Holotype**, HSC; GenBank KT074359 (mtSSU), KT074357 (LSU), KT074361 (RPB2)).

Etymology — named for Alissa Allen, who first discovered this species.

Basidiomata with the pileus and stipe initially covered with silvery-whitish evanescent layer of fibrils that forms squamules over the pileus surface, small pointed squamules on the pileus margin, and longitudinally arranged appressed fibrils on the stipe. When fresh, the stipe fibrils can be rubbed off revealing a dark brownish fibrillose layer on the stipe surface. In older basidiomata, this layer first disappears from the pileus before eventually disappearing on the

Plate 1. *Pouzarella alissae* (HSC DLL10490, holotype): A. basidiomata (Photo by Noah Segal); B. cross section through strigose stipe base; C. longitudinal section through strigose stipe base. Scale bars: A = 8 mm; B, C = 20 µm.

stipe. In silica gel-dried basidiomata, this layer is absent when sections of the basidiomata are viewed under the compound microscope.

Pileus 8–30 mm broad, 2–7 mm high, conic to campanulate, becoming convex to broadly convex, then plane, and finally uplifted; initially lacking an umbo or obscurely umbonate, broadly umbonate when mature; opaque (not

translucent and thus not translucent-striate), dull, scurfy or tomentulose on disc, innately fibrillose elsewhere; margin incurved then decurved and eventually plane; color overall dark brownish grey (4–5F2–3) fading to dark brown (5F3–4); flesh ≤1.5 mm above the stipe, pale greyish brown. Lamellae 6–12 mm long, 1.25–4 mm deep, initially adnexed and sigmoid and later narrowly to broadly adnate (at times with a small subdecurrent tooth), subdistant to distant, moderately broad, pale greyish-tan when young, maturing dark greyish-brown (5F2) with smoky greyish brown (5–7F5) spots and blotches, when fully mature with a pinkish cast from spores; edge fairly thick, at first concolorous and then developing whitish areas with spore maturation; lamellulae 3–4 between lamellae in two series [one always long (4–8 mm) and one always short (1–2.5 mm)]. Stipe 33–88 mm long, apex 1–2.5 mm diam. and base 1.25–4.5 mm diam., cylindrical, equal when young and enlarged at the base when mature, very finely pruinose at the apex and innately and appressed-fibrillose elsewhere, in very scattered places the fibrils separating and becoming matted in clumps; hairs or setae absent; basal area decidedly strigose up to 7–8 mm from substrate, brownish with reddish tones; basal tomentum also present, whitish (in some basidiomata developing orangish areas). Taste and Odor not distinctive. Spore deposit pinkish to dingy salmon-tan.

Basidiospores with 5–6 distinct angles in side and profile views, heterodiametric in side, profile, dorsal, and ventral views, 9–12 × 6–8 µm (m = 10.2 ± 0.8 × 7.2 ± 0.5 µm; E = 1.2–1.8; Q = 1.4 ± 0.1; n/1 = 40). Basidia cylindro-clavate, 34–47 × 8–13 µm (m = 41.2 ± 3.5 × 9.8 ± 1.5 µm; E = 3.2–6.0; Q = 4.3 ± 0.7; n/1 = 10), 4-sterigmate; aborted basidia abundant on the lamellar edge and face, wall thickening at first after which the entire contents become reddish brown in 10% NH_4OH or 3% KOH, cylindro-clavate, 21–39 × 4–11 µm (m = 29.9 ± 4.2 × 6.5 ± 1.6 µm; E = 3.1–8.3; Q = 4.8 ± 1.3; n/1 = 20). Lamellar edge heterogeneous. Cheilocystidia (leptocystidia) thin-walled, when present scattered (but often absent on some lamellae), colorless or staining reddish brown in alkali solutions, versiform (cylindro-clavate, clavate, obclavate), 34–62 × 10.5–16.4 µm or lageniform with a short to medium neck or rostrate-ventricose, 46–656 × 13–23.5 µm (including the neck or rostrum), rostrum typically short to medium (rarely longer), 3.1–7.0(–17.5) × 3.0–3.5 (–6.0) µm (n/1 = 4)). Pleurocystidia colorless, uncommon and very scattered, obclavate, acuminate-ventricose, lageniform, or rostrate-ventricose, 40–42.5 × 12–17 µm (including rostrum, which is 6.4–7.0 µm long and 2.7–7.0 µm diam.). Lamellar tramal hyphae subparallel, 125–564 × 9.2–42 µm (E = 9.2–19.1; n/1 = 10). Pileipellis composed of entangled hyphae, erect and resembling a trichodermium on the disc, semi-erect around the disc, becoming

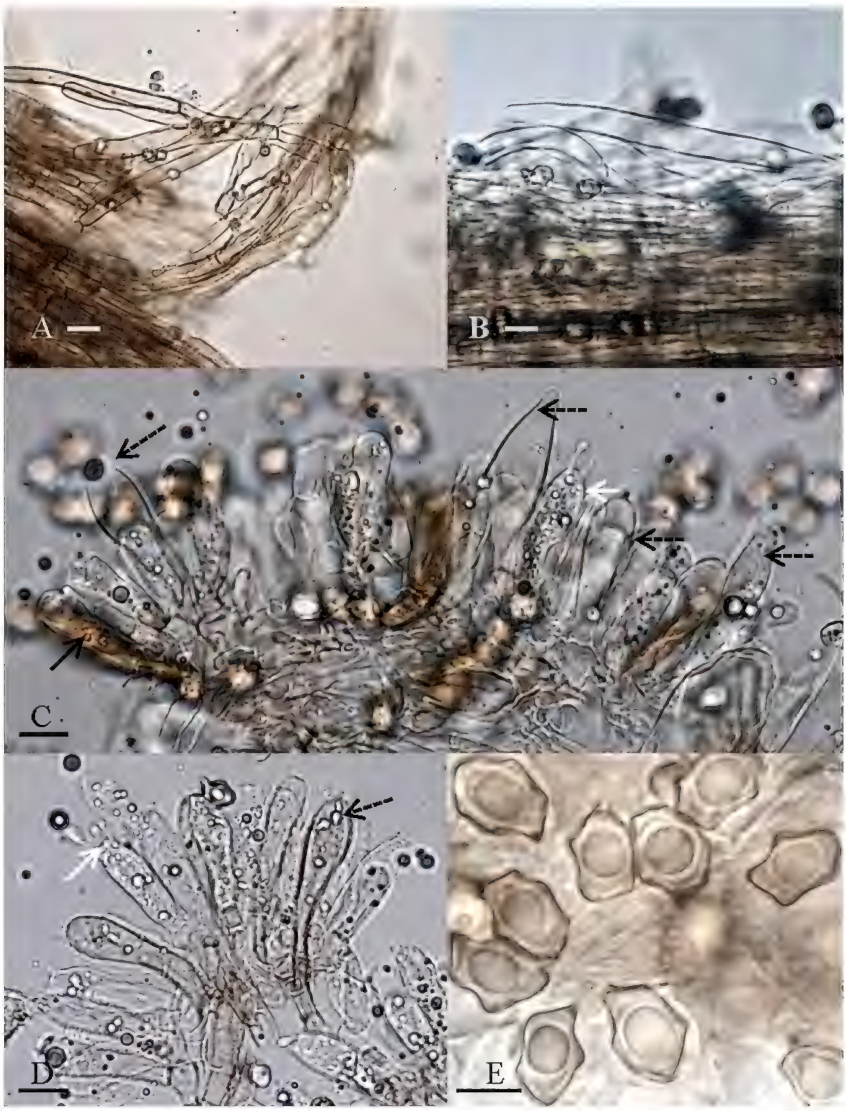

Plate 2. *Pouzarella alissae* (HSC DLL10490, holotype): A. pileipellis radial section from disc; B. stipitipellis with caulocystidium (longitudinal section from mid-stipe); C. basidium (white arrow), aborted basidium filled with dark pigment (black solid arrow), and cheilocystidia (dotted arrows); D. basidium (white arrow) vs. aborted basidium (dotted arrow); E. basidiospores. Scale bars: A, B = 12 µm; C = 15 µm; D = 10 µm; E = 7 µm.

prostrate towards the margin; PILEOCYSTIDIA (terminal cells) on or around the disc cylindro-clavate with a rounded apex 93–184 × 9–18 µm (*m* = 131.9 ± 31.1 × 12.6 ± 2.4 µm; E = 8.6–14.3; Q = 10.6 ± 2.1; n/1 = 11), towards and at the margin/edge longer and tapered, 166–218 × 14–17 µm. PILEAL TRAMAL HYPHAE subparallel and entangled, 49–310 × 3–30.5 µm (*m* = 152.2 ± 71.6 × 13.7 ± 9.4 µm; E = 4.9–46.3; Q = 15.9 ± 12.3; n/1 = 13). STIPITIPELLIS at the apex composed of hymenial elements (= pruinose), below the apex hyphae primarily prostrate, 2–4 cells deep but occasionally out-turned in some areas; caulocystidia (terminal cells) cylindro-clavate and similar in shape to the pileocystidia, 67–136 × 9–14 µm (m = 89.4 ± 24.4 × 11.2 ± 1.9 µm; E = 5.8–12.2; Q = 8.2 ± 2.5; n/1 = 6). STIPE TRAMAL HYPHAE nearly parallel, somewhat entangled, 9–67 µm diam. (n/1 = 7). OLEIFEROUS HYPHAE rare in the pileal trama. BRILLIANT GRANULES scattered to somewhat abundant in the basidia. LIPOID GLOBULES absent. PIGMENTS brownish, cytoplasmic and parietal on the apical 1–4 cells in the pileus and stipe suprapelli, encrusted as rings in the pileus and stipe subpelli, and decidedly encrusted on all tramal hyphae. Clamp connections absent.

ECOLOGY & DISTRIBUTION — Solitary, in clumps or loose clusters in wet duff under *Acer circinatum* Pursh and *Polystichum munitum* (Kaulf.) C. Presl in Prairie Creek Redwoods State Park.

COMMENTS — Morphologically, *P. alissae*, *P. araneosa*, *P. celata* Mazzer, *P. versatilis*, and *Entoloma subaraneosum* Xiao L. He & T.H. Li are placed in *Pouzarella* sect. *Versatiles* Mazzer because of their innately fibrillose pileus and stipe, thin-walled hymenial cystidia, and basidiospores that average <13 µm in length. *Pouzarella araneosa* and *E. subaraneosum* can be differentiated from *P. alissae* by their longer spores (9.5–15 µm for *P. araneosa*; 9.5–13 µm for *E. subaraneosum*), longer cheilocystidia (45–100 µm) with much longer necks, and lack of pleurocystidia (He et al. 2013; Mazzer 1976; Noordeloos 1992, 2004).

In North America, *P. celata,* described from Michigan, and *P. versatilis,* described from Europe (Noordeloos 1979, 1992, 2004; as *Entoloma versatile* (Gillet) M.M. Moser) and reported from western North America including California (Mazzer 1976) and Washington (Largent 1994), are similar in size and also produce dark brown colored basidiomata. Both species can be distinguished from *P. alissae* by their abundant and longer cheilocystidia and pleurocystidia and absence of silvery white evanescent fibrils and squamules on the pileus. *Pouzarella celata* also differs by its longer (11–14 µm) basidiospores and glabrous pileus surface that is streaked by innate silky grey fibrils while *P. versatilis* is distinguished by its abundant pleurocystidia, olivaceous green

tints or olivaceous brown pileus color (Noordeloos 2004, Largent 1994), and the metallic luster of the dried pileus (Mazzer 1976).

Pouzarella araneosa, reported from California under *Acer circinatum,* consisted of one basidiome with a missing stipe base (Largent 1994). The collection differs from *P. alissae* and *P. araneosa* by the absence of a silvery fibrillose layer, its medium dark brown (5–6F4) pileus, its translucent-striate pileal margin, and its broader and longer cheilocystidia; until more material is obtained, the diagnosis of the collection formerly reported as *P. araneosa* remains doubtful.

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The discovery of *Syncephalis obliqua* (*Zoopagomycotina, Zoopagales*) in the Neotropics

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Abstract — During an investigation on coprophilous fungi from Brazil, several sporangiophores of a mycoparasitic fungus were recovered from horse dung collected in Recife, Pernambuco, Northeastern Brazil, and identified as *Syncephalis obliqua*. This is the first record of the species in the Neotropics, as well as in a host growing on herbivore dung, and its second world record. A detailed description and high quality images are provided, along with an identification key to all *Syncephalis* species recorded in Brazil.

Key words — *Piptocephalidaceae*, taxonomy

Introduction

Syncephalis was first described by van Tieghem & Le Monnier (1873), and c. 60 species are accepted (Ho & Benny 2008, Kirk et al. 2008). The genus includes haustorial mycoparasites of *Mortierellales*, *Mucorales*, or (less commonly) ascomycetes, forming coenocytic aerial hyphae in or over the host, merosporangiophores with basal rhizoids, and usually with apical fertile vesicles bearing several one- to multi-spored merosporangia. Zygospores, when present, are globose to subglobose, ornamented, with apposed suspensors, possibly adorned with a multi-lobed vesicle (Benny 2011). Most records are associated with mucoraceous hosts in soil and dung (Santiago et al. 2010).

In Brazil, only seven species have previously been recorded: *S. aggregata* A.L. Santiago & Benny, *S. asymmetrica* Tiegh. & G. Le Monn., *S. clavata* H.M. Ho & Benny, *S. cornu* Tiegh. & G. Le Monn., *S. penicillata* Indoh, *S. sphaerica* Tiegh., and *S. tengi* S.H. Ou (Trufem 1984, Trufem & Viriato 1985, Melo et al. 2011).

Syncephalis obliqua was described by Ho & Benny (2008) parasitizing a *Mucor* species from soil samples collected in Taiwan. Here we present the second world record of *S. obliqua*, identified as a mycoparasite of coprophilous ascomycetes on herbivore dung from Brazil and recorded for the first time in the Neotropics.

Materials & methods

Samples of cattle, goat and horse dung collected in clean plastic bags on the campus of Universidade Federal Rural de Pernambuco, Recife (8°00′54″S 34°56′59″W) were taken to the laboratory and incubated in moist chambers (9 cm diam. Petri dishes with a moistened disk of filter paper) at room temperature (28 ± 2°C) for at least 20 days under alternating natural light and dark periods. The specimens were observed directly from the substrata under a Leica EZ4 stereomicroscope; sporangiophores were mounted in slides with tap water, lactophenol plus cotton blue, or Polyvinyl-Lacto-Glycerol and examined with the aid of a Leica DM500 light microscope. Specimens were identified based on Benny (2011), Ho & Benny (2008), and Santiago et al. (2011). A careful literature review and survey of Brazilian herbaria were performed in order to access information regarding older *Syncephalis* records. High quality images were captured with a QImaging QColor 3 digital camera mounted on an Olympus BX51 compound microscope using differential interference or phase contrast microscopy. Permanent slides were deposited in Herbário Padre Camille Torrend, Departamento de Micologia, Universidade Federal de Pernambuco, Recife, Brazil (URM). Additional information regarding deposit records can be accessed at the "INCT - Herbário Virtual da Flora e dos Fungos" database website (http://inct.florabrasil.net/).

Taxonomy

Syncephalis obliqua H.M. Ho & Benny, Bot. Studies 49: 45 (2008). PLATE 1

SOMATIC HYPHAE hyaline, 1.5–2 µm wide, immersed in substrata or on host hyphae, weakly cyanophilous. SPORANGIOPHORES numerous, isolated, erect, simple, cylindrical, hyaline, smooth to slightly punctate, arising from the host hyphae or from the dung, broader at the base, 650–710 µm long, 15–17.5 µm diam., narrowing gradually towards the apex, 7.5–10 µm diam. just below the vesicle. RHIZOIDS well developed, hyaline to weakly pigmented, branching to form horizontal primary rhizoids that branch once or twice, septate. APICAL VESICLES globose to obovoid, hyaline, persistent or collapsing, cyanophilous, 62.5–77.5 × 52.5–62.5 µm, with about half of its apical surface covered by merosporangia, leaving conspicuous denticles on the surface after release. MEROSPORANGIA 4–6-spored, pedunculate, unbranched, thin-walled, hyaline, cylindrical, obliquely placed on the vesicle, usually detaching in mounts. MEROSPORES cylindrical to doliiform, rounded on both ends or eventually with one end truncate and the other rounded, hyaline, smooth, 5.5–6.5 × 2.5–3 µm. ZYGOSPORES not observed.

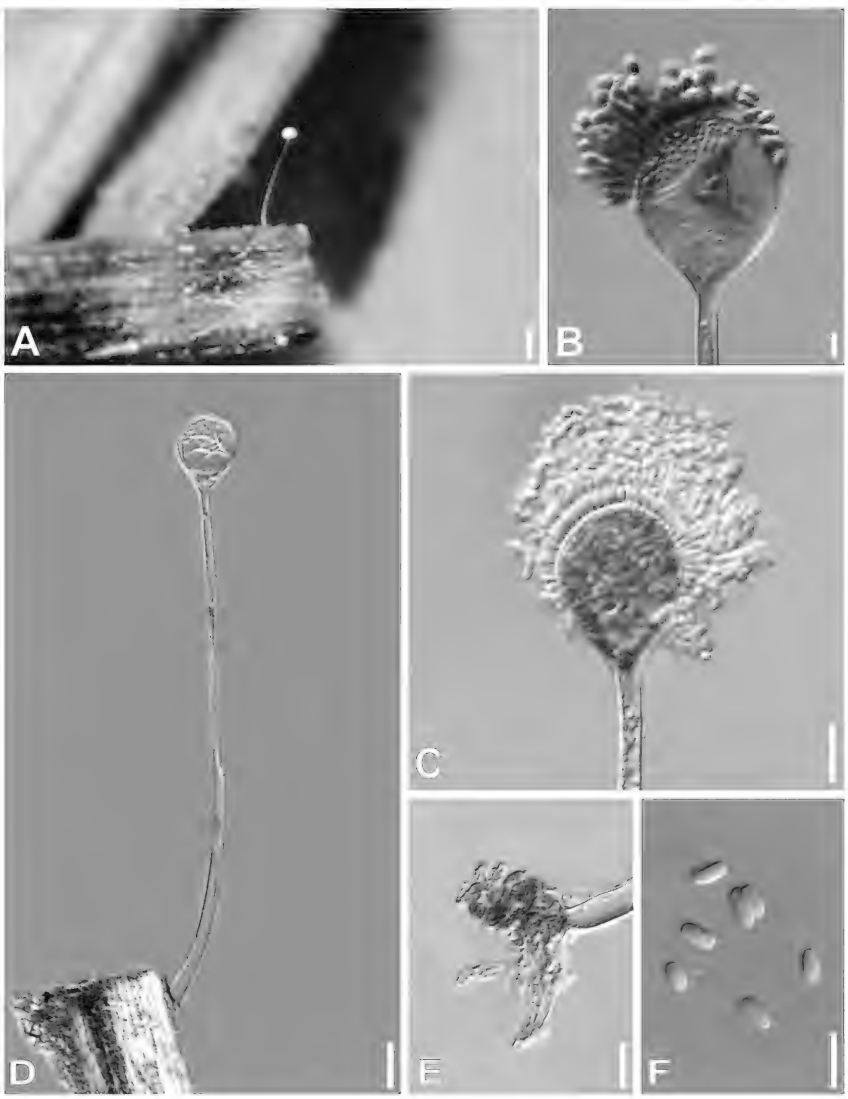

Plate 1. *Syncephalis obliqua* (URM 87589a): A. sporangiophore growing on host immersed on dung; B. apical vesicle of a young sporangiophore, strongly cyanophilous, showing the obliquely placed merosporangia on the left; C. apical vesicle in a mature sporangiophore, with several visible multispored merosporangia; D. sporangiophore after merosporangia release, showing conspicuous denticles on the upper half of the vesicle; E. rhizoids; F. released merospores. Scale bars: A = 250 µm; B = 10 µm; C = 50 µm; D, E = 25 µm; F = 15 µm.

Habitat: Parasitic on *Ascobolus immersus* Pers. (*Pezizales*) and *Zopfiella longicaudata* (Cain) Arx (*Sordariales*) growing on horse dung.

Material examined: **BRAZIL. Pernambuco**, Recife, Dois Irmãos, Universidade Federal Rural de Pernambuco, IX.2011, R.F.R. Melo s.n. (URM 87588); XI.2014, R.F.R. Melo s.n. (URM87589a–f).

Distribution: Brazil and Taiwan. This is the first record from Brazil.

Notes: The main distinguishing feature of *S. obliqua* is the oblique placement of the merosporangia on the apical vesicle. It differs from *S. plumigaleata* by the larger merospores (6.5–10 × 3.5–4.5 µm; Embree 1965) and from *S. fuscata* (which produces similar obliquely placed merosporangia) in spore shape, ornamentation, and size (Kuzuha 1973). Our Brazilian specimens show a close morphological similarity with the descriptions of the Taiwanese material by Ho & Benny (2008). However, some merospores are slightly truncate at one end, which is not noted in the original description. Most specimens observed on incubated dung produced several sporangiophores (usually more than 100 per 5 g of dung) and persisted for 4–5 weeks. Although some sporangiophores arose from *Ascobolus immersus* apothecia and *Zopfiella longicaudata* cleistothecia growing on dung, clear signs of direct parasitism were not observed.

Key to *Syncephalis* spp. recorded from Brazil

1. Sporangiophores single, strongly curved ..... *S. cornu*
   Sporangiophores single or in clusters, erect or slightly curved 2
2. Sporangiophores with irregular swellings, rarely branched, forming dense tufts ..... *S. aggregata*
   Sporangiophores without swellings, unbranched, single 3
3. Vesicle truncate; merosporangia cylindrical at the base and unilaterally branched ..... *S. penicillata*
   Vesicle and merosporangial base not as above 4
4. Merosporangia branched once at the base, the first spore being cordate, irregularly shaped 5
   Merosporangia unbranched 6
5. Vesicle asymmetric; merospores 4–8 × 2–4 µm ..... *S. asymmetrica*
   Vesicle symmetric; merospores 7.5–12.5 × 3.5–5 µm ..... *S. clavata*
6. Merosporangia obliquely placed at the vesicle ..... *S. obliqua*
   Merosporangia equally placed at the vesicle 7
7. Merosporangia 2–8 spored; merospores 6-8 × 8 µm long ..... *S. tengi*
   Merosporangia 3–4 spored; merospores 5-5.1 × 11-12 µm long ..... *S. sphaerica*

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Two new records of *Agaricus* spp. from Ethiopia

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Abstract — *Agaricus xanthodermus* and *A. xanthodermulus* are recorded as new to Ethiopia based on morphological and molecular data. A description of *A. campestris* (previously reported from Ethiopia) is also provided. The specimens were collected from Menagesha and Holeta sites located in the Wolmera District of Oromia Special Zone surrounding Addis Ababa. Morphological identification of the specimens was supported by nrDNA ITS sequence analysis. All three species are described in detail and illustrated based on the collections from Ethiopia.

Key words — *Agaricaceae*, *Basidiomycota*, taxonomy, highland, mycota

Introduction

Agaricus L. is a large and important genus encompassing many edible and few poisonous mushrooms, with possibly 200–400 (Bas 1991, Kirk et al. 2008, Zhao et al. 2011) to possibly over 400 (Thongklang et al. 2014) species worldwide. *Agaricus* species are generally distinguished from other members of the family *Agaricaceae* by their small to large basidiomata, white or brown pileus, annulus and central stipe, free lamellae that are whitish or pinkish when young and chocolate-brown to dark brown when mature, and smooth dark basidiospores (Cappelli 1984). Studies have shown that the genus is monophyletic (Geml et al. 2004) and contains eight recognized sections (Parra 2008), some of which have been phylogenetically reconstructed, e.g., *A.* sect. *Bivelares* (Kauffman) L.A. Parra and *A.* sect. *Xanthodermatei* Singer (Challen et al. 2003, Kerrigan et al. 2005, Thongklang et al. 2014). *Agaricus* species are generally found in pastures among grass and in mixed forests (Bas 1991).

TABLE 1. ITS sequences of *Agaricus* and outgroup species used in the molecular analysis.

SPECIES	VOUCHER/STRAIN	ORIGIN	GENBANK*
Agaricus aff. *endoxanthus*	ZRL3095	Thailand	JF691554
A. aridicola	CA101	France	JF797195
A. arvensis	CA640	France	JF797194
A. augustus	CA590	France	JF797193
A. benesii	LAPAG283	France	JF797179
A. bernardii	strain ARP173	USA	AF432880
A. bitorquis	strain RWK1462	USA	AF432898
A. bohusii	LAPAG531	Czech Republic	JF797180
A. bresadolanus	strain CA177	France	DQ185570
A. brunneolus	CA490	France	JF797203
A. californicus	strain RWK 1936	USA	DQ182510
A. campestris	strain W1H	USA	DQ182533
	Strain B9	China	JX434655
	strain H2	India	KM609406
	strain AGRK111	China	FJ223222
	HMAS272461	Ethiopia, Menagesha	**KP229419**
	HMAS272462	Ethiopia, Holeta	**KP229420**
A. caribaeus	F2530	Martinique	JF727856
A. cupreobrunneus	LAPAG 322	Spain	JQ824136
	strain CA 87	USA	DQ182532
A. fissuratus	strain WC777	Denmark	AY484683
A. gennadii	CA387	France	JF797188
A. langei	strain WC784	USA	AY484699
	LAPAG141	France	JF797181
A. laskibarii	LAPAG115	USA	*AY943975*
A. moelleri	strain CA31	France	AY899263
	strain CA156	France	AY899264
A. parvitigrinus	strain CA157	France	AY899266
	strain CA158	France	AY899267
A. pseudolutosus	LAPAG77	Spain	JF727868
A. silvaticus	LAPAG341	France	JF797178
A. viridopurpurascens	Horak68/79	New Zealand	JF514525
A. xanthodermus	strain CA15	France	AY899271
	strain CA161	France	AY899272
	strain W3I	England	DQ182534
	strain CA236	France	DQ185564
	strain CA6	France	DQ185563
	HMAS272456	Ethiopia, Menagesha	**KP229414**
	HMAS272457	Ethiopia, Holeta	**KP229415**
A. xanthodermulus	strain CA160	France	AY899273
	strain CA174	France	AY899274
	strain CA204	France	AY899276

Table 1, concluded

(*A. xanthodermulus*)	LIP CA160	France	NR119528
	strain CA188	France	AY899275
	HMAS272459	Ethiopia, Holeta	**KP229416**
	HMAS272460	Ethiopia, Menagesha	**KP229417**
A. xanthosarcus	Goossens5415	Belgium	JF514523
Hymenagaricus ardosiicolor	LAPAF9	Togo	JF727840
Heinemannomyces splendidissimus	ecv3586	Thailand	HM488760

* Sequences produced in this study in bold.

Pegler (1977) noted that agaric mycota in East Africa is extremely rich both in the range of genera and in the number of species. However, large parts of this region remain mycologically unexplored. Although Ethiopia is known to have highly diverse flora and fauna (Friis & Sebsebe 2001) but the diversity, ecology, and distribution of macrofungi is so far poorly explored.

Macrofungal diversity in general and *Agaricus* species in particular have not been a focus of taxonomic investigation in Ethiopia. Only a few reports on macrofungi in Ethiopia have been published (Abate 1995, 1999). Abate (1999), who cited *Agaricus campestris* as the dominant mushroom in the grazing areas on the highland (2000–3000 m) plateau in southern (Bale), central (Debreberhan), and northwestern (Gojam) Ethiopia, appearing in a large numbers during the middle of the rainy season (July and August) did not provide a detailed morphological description or any molecular data of the species.

As the first report of the on-going work on macrofungal diversity in Ethiopia, we present detailed morphological descriptions and molecular analyses of three *Agaricus* species including two new records, *A. xanthodermus* and *A. xanthodermulus,* for Ethiopia in addition to the previously cited *A. campestris*. This report will provide a baseline for future taxonomic, ecological, and economic studies on Ethiopian *Agaricus* species.

Materials & methods

Collection sites

Specimens were collected from Menagesha and Holeta, Wolmera district, in the Oromia Special Zone surrounding Addis Ababa, the capital of Ethiopia, during June–September, 2012–2013. Menagesha (9°02′N 38°35′E, 2500–3300 m a.s.l.) comprises forests and grazing areas. Holeta (9°03′N 38°30′E, 2391 m a.s.l.) contains mainly settlement and grazing areas.

Morphological observation

Distinguishing macroscopic features and microhabitat were noted for each specimen in the field. Microscopical observations were made from dried specimens mounted in 5% KOH and stained with 1% aqueous Melzer's reagent and Congo red. At least 20

basidiospores were measured from each specimen. Chemical testing involved the application of 20% KOH on the pileus surface, with negative = no colour change and positive = change to yellow or orange. Dried specimens are deposited in the Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, Ethiopia, and duplicates are preserved in the Fungarium, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS). The specimens were identified morphologically according to Pegler (1977), Pegler & Piearce (1980), Singer (1986), and Callac & Guinberteau (2005). Abbreviations: RSK = Rediet Sitotaw Kebede.

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from dried specimens using the modified CTAB method described by Yao et al. (1999). The fungal universal primer pairs ITS5/ITS4 were used to amplify the internal transcribed spacer (ITS) region and LR0R/LR5 to amplify the nuclear larger subunit RNA (LSU) region (White et al. 1990). The PCR reaction mixture was comprised 25 µL Taq PCR MasterMix, 0.5 µL of 10 µM each primer, 1 µL diluted DNA template, and RNase-Free water to bring the total volume to 50 µL. The PCR conditions were as follows: 94 °C for 5 min, followed by 35 cycles of 95 °C for 1min, 53 °C for 1min, 72 °C for 1 min and final extension step of 72 °C for 10 min on a GenAmp PCR System 9700 thermocycler (Vers. 3.03).

PCR products were sequenced from both directions using the same primers on an Applied Biosystems 3730 Analyzer™ by the Beijing Genomics Institute (Beijing, China). Sequence ends were manually edited and assembled using DNAStar Lasergene SeqMan™ II vers. 6.1. Sequences were then aligned using Clustal W algorithm (Thompson et al. 1994) and manually edited with BioEdit vers. 7.1.9 (Hall 1999).

Phylogenetic analysis

ITS sequences from the Ethiopian *Agaricus* collections were aligned with 43 closely related ITS sequences from GenBank representing the eight *Agaricus* sections (Zhao et al. 2011) (Table 1). *Hymenagaricus ardosiicolor* and *Heinemannomyces splendidissimus* were selected as outgroup taxa since these species consistently formed a sister clade to the monophyletic *Agaricus* (Zhao et al. 2011). The datasets were analysed using PAUP vers. 4.0b8 (Swofford 2002). Maximum parsimony (MP) analyses were conducted with 1000 replicates of random sequence addition followed by tree-bisection reconnection branch swapping. All characters were equally weighted, and gaps were treated as missing data. Bootstrap proportions (BP) were calculated using analyses of 1000 replicates with five replicates of random sequence addition.

Results of phylogenetic analyses

ITS sequences from the six Ethiopian specimens were submitted to GenBank as KP229414–229417, 229419, and 229420. (Analysis of the LSU sequences also obtained from the same specimens and deposited in GenBank as KP331526–KP331530 will be presented in a future publication.)

A total of 49 ITS sequences (Table 1) were aligned for the phylogenetic analysis. The dataset generated a MP tree with 709 total characters, of which 439 were constant and 185 parsimony informative sites. Our ITS sequence analysis

supported the eight major *Agaricus* sections, and MP analyses confirmed the placement of our six sequences in two well-defined sections (FIGURE 1).

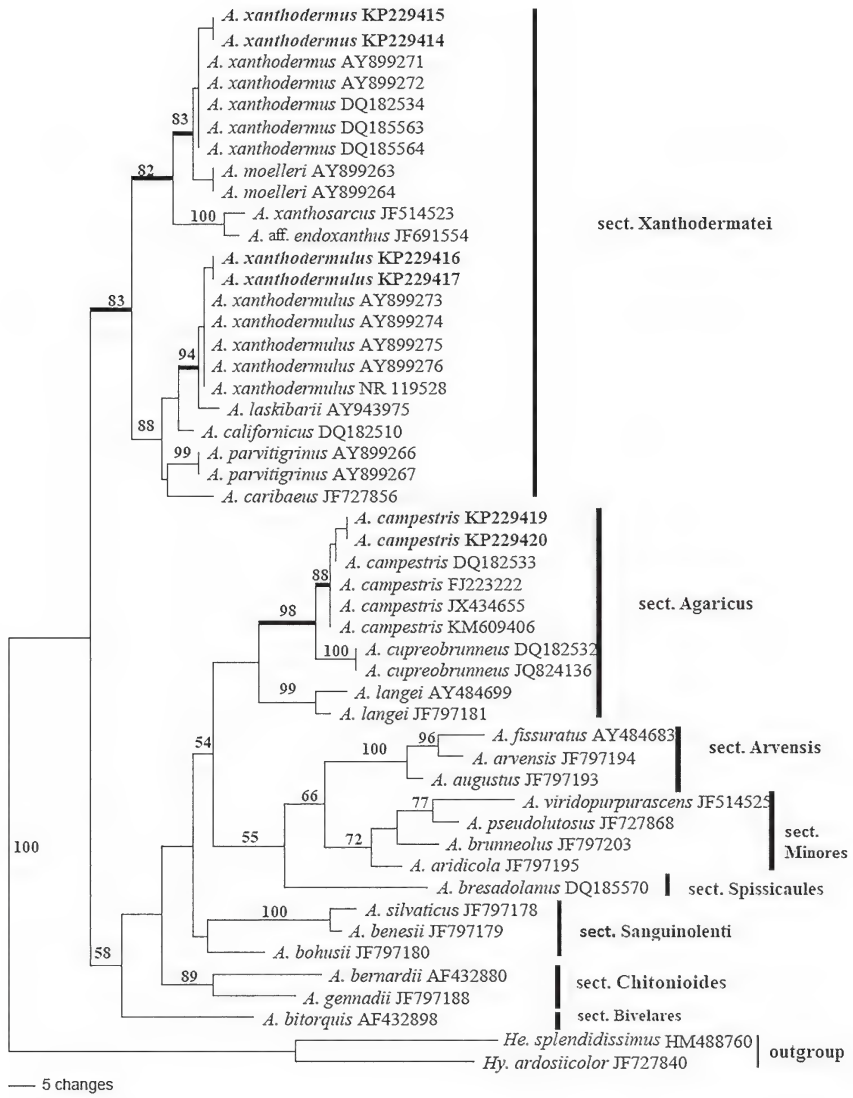

FIGURE 1. MP tree based on nrDNA ITS sequences. The species in bold were sequenced by the authors. Bootstrap numbers greater than 50% are included above branches; analysis performed in PAUP* using 1000 bootstrap replicates.

Two sequences (KP229419, KP229420) fell within *A.* sect. *Agaricus*, where they grouped with *A. campestris* sequences retrieved from GenBank with 88% bootstrap support. The other four sequences fell within *A.* sect. *Xanthodermatei,* two (KP229416, KP229417) grouping with *A. xanthodermulus* with 94% bootstrap support for the clade (containing *A. xanthodermulus* and *A. laskibarii*) and two (KP229414, KP229415) grouping with *A. xanthodermus* with 83% bootstrap support for the clade (containing *A. xanthodermus* and *A. moelleri*).

Taxonomy

Agaricus campestris L., Sp. Pl. 2: 1173 (1753). FIG. 2

PILEUS 4–8 cm diam., at first subglobose to hemispherical, becoming convex, broadly convex to plano-convex, sometimes slightly umbonate; surface white to cream-color, sometimes slightly ochraceous, with grayish to pale ochraceous fibrils or fibrillose scales, dry, sometimes silky; margin incurved at first, finally expanding to decurved, with remnants of partial veil attached. LAMELLAE free, ≤5 mm wide, initially pinkish, then pinkish brown to cinnamon-brown, finally dark brown, crowded, with lamellulae. STIPE 3–8 × 0.5–1(–1.5) cm, central, cylindrical or slightly tapering toward base; surface white and silky above the annulus, white to brownish with whitish appressed fibrils below; solid to soft, fibrous. CONTEXT white or whitish, fleshy, soft. ANNULUS thin, white, membranous, superior. ODOR and TASTE pleasant. BASIDIOSPORE DEPOSIT dark brown. CHEMICAL REACTION 20% KOH negative.

BASIDIOSPORES 5–8.5 × 3.5–5.5 µm, avl × avw = 6.5 × 4.5 µm, Q = 1.4–1.5, avQ = 1.45, ellipsoid to ovoid, pale brown to brown, inamyloid, smooth, thick-walled. BASIDIA 18–24 × 6–8 µm, clavate, 4-spored, thin-walled, subhyaline to brownish. LAMELLA EDGE heterogeneous, with sterile cells, 14–22 × 5–7.5 µm, clavate, thin-walled, subhyaline to brownish. CHEILOCYSTIDIA not found. SUBHYMENIUM ≤10 µm wide; hyphae narrow, branched, hyaline, thin-walled, 2–5 µm diam. HYMENOPHORAL TRAMA regular, 80–120 µm wide, hyphae hyaline, thin-walled, 4.0–20 µm diam. PILEIPELLIS a radially aligned repent epicutis; hyphae 3.5–10 µm diam., cylindrical, branched, hyaline to subhyaline, thin-walled. PILEUS TRAMA hyphae 4.5–20 µm diam., cylindrical to inflated, hyaline, thin-walled. ANNULUS hyphae 3.5–8.5 µm diam., cylindrical, hyaline to subhyaline, thin-walled. CLAMP CONNECTIONS absent.

HABITAT: Scattered in pastureland.

SPECIMENS EXAMINED: **ETHIOPIA**, **OROMIA SPECIAL ZONE**, **Wolmera District**, Holeta, on grazing land, 26 June 2012, RSK32 (HMAS 272461; GenBank KP229419); Menagesha, Menagesha suba forest, 10 July 2013, RSK33 (HMAS 272462; GenBank KP229420).

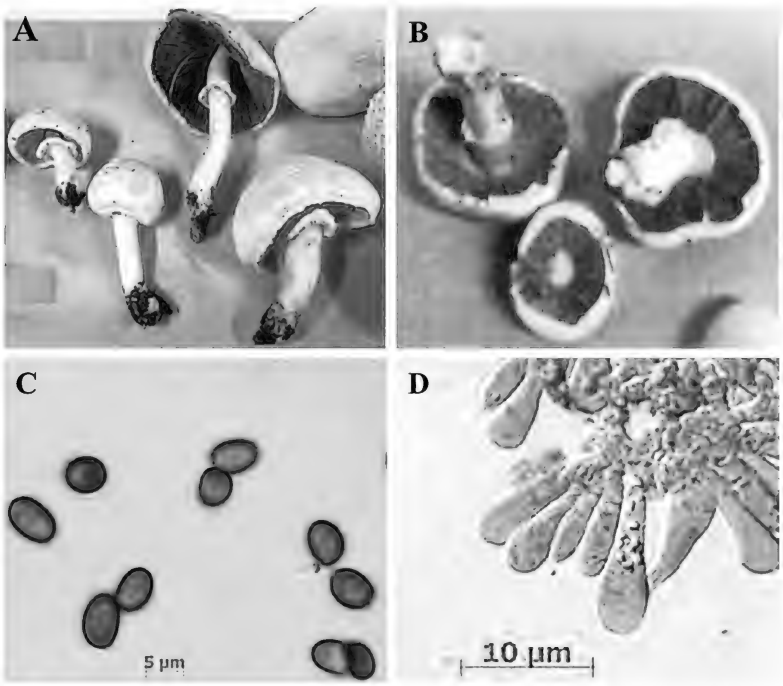

FIGURE 2. *Agaricus campestris*:
A. basidiomata (HMAS 272461); B. basidiomata (HMAS 272462);
C. basidiospores (HMAS 272461); D. cystidia (HMAS 272462).

REMARKS—Although *A. campestris* was first reported from Ethiopia by Abate (1999), we have included this species to provide detailed morphological and molecular data from Ethiopian specimens. As noted by Abate (1999), *A. campestris* was very common on the grazing area of the highland plateau of Ethiopia and we found it to be fairly common and widespread in the grazing area (above 2300 m) right after the beginning of the rainy season. Our *A. campestris* specimens share similar morphological traits of the *A. campestris* complex: a white, subglobose to broadly convex pileus, initially pinkish then pinkish brown and finally dark brown lamellae, a membranous white annulus, a negative KOH reaction, and a mild taste and odor (Pegler 1977, Mitchell & Walter 1999, Kerrigan et al. 2005).

Agaricus xanthodermulus Callac & Guinb., Mycologia 97: 421 (2005). FIG. 3

PILEUS 5–7 cm diam., cuboidal at first, then conico-convex to broadly convex, finally expanding to plano-convex, slightly umbonate at center; surface

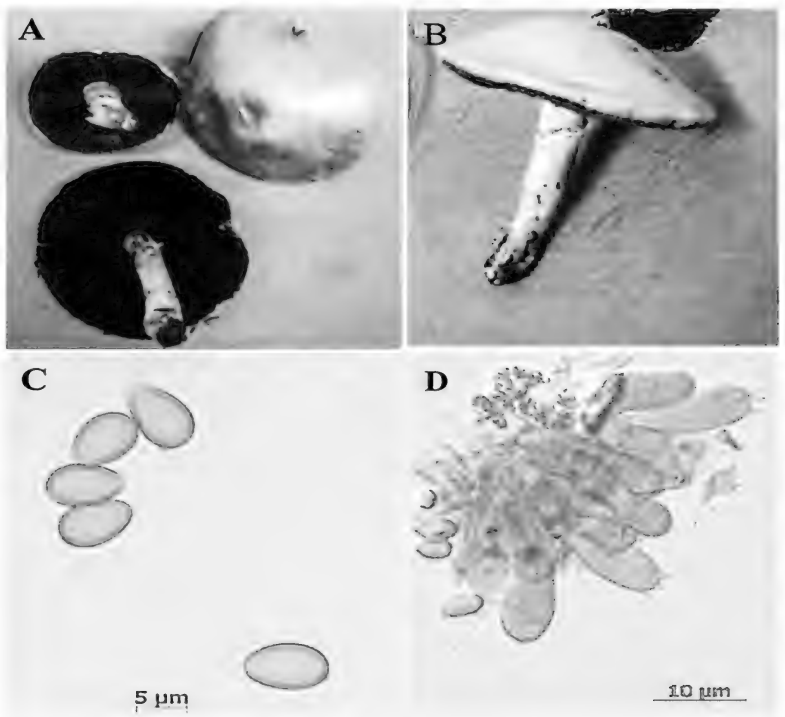

Figure 3. *Agaricus xanthodermulus*:
A. basidiomata (HMAS 272459); B. basidiomata (HMAS 272460);
C. basidiospores (HMAS 272459); D. basidia (HMAS 272460).

white to grayish, becoming yellow when rubbed, sometimes squamulate, dry; margin incurved at first, then decurved. Lamellae free, ≤5 mm wide, pinkish at first, then pinkish brown to brown, finally dark brown, crowded, with lamellulae. Stipe 4–8 × 0.5–1 cm, central, cylindrical with slightly bulbous base; surface white to whitish, becoming yellow when rubbed; solid at first, then soft, finally hollow, and fibrous in old specimen. Context white or whitish, becoming light yellow when cut, fleshy. Annulus white to whitish, membranous, superior. Basidiospore deposit dark brown. Taste and Odor unpleasant. Chemical reaction with 20% KOH positive.

Basidiospores 5–7 × 4–5.5 µm, avl × avw = 6.0 × 4.7 µm, Q = 1.25– 1.27, avQ = 1.26 ellipsoid, pale brown to brown, inamyloid, smooth, thick-walled. Basidia 18–27 × 6–8 µm, clavate, 4-spored, thin-walled, subhyaline to brownish. Lamella edge heterogeneous, with sterile cells, 15–24 × 5–7 µm, clavate, thin-walled, subhyaline to brownish. Cheilocystidia rare,

18–27 × 7–11 µm, clavate to pyriform, thin-walled, hyaline. Subhymenium ≤12 µm wide; hyphae narrow, branched, hyaline, thin-walled, 3.5–5 µm diam. Hymenophoral trama regular, 80–120 µm wide; hyphae hyaline, thin-walled, 5–20 µm diam. Pileipellis a repent radially aligned epicutis; hyphae thin-walled, 4–10 µm diam., cylindrical, branched, hyaline to subhyaline. Pileus trama hyphae 5–20 µm diam., cylindrical to inflated, hyaline, thin-walled. Annulus hyphae 4–9 µm diam., cylindrical, hyaline to subhyaline, thin-walled. Clamp connections absent.

Habitat: Solitary, grazing land.

Specimens examined: **ETHIOPIA, Oromia Special Zone, Wolmera District**, Holeta, on grazing land, 10 July 2013, RSK12 (HMAS 272459; GenBank KP229416); Menagesha, Menagesha suba forest, 10 July 2013, RSK12 (HMAS 272460; GenBank KP229418).

Remarks—*Agaricus xanthodermulus,* described recently by Callac & Guinberteau (2005) from France, differs from other members in *A.* sect. *Xanthodermatei* by its small size (maximum cap diameter 6 cm) and larger spores (6.7–7.8 × 4.5–5.5 µm). The Ethiopian material generally agreed with the original description, although its slightly larger pileus and slightly smaller spores brought it closer to the type specimen of *A. xanthodermus* (with a 6.0–12 cm diam. pileus and 4.0–5.5 × 3.0–4.0 µm spores; Callac & Guinberteau 2005). Kerrigan et al. (2005) suggested that *A. xanthodermulus* might represent a small form of *A. xanthodermus.* The ITS sequences show only two nucleotide differences (at positions 120 and 537) between our collections and *A. xanthodermulus* from France.

Agaricus xanthodermus Genev., Bull. Soc. Bot. Fr. 23: 32 (1876) Fig. 4

Pileus 6–12 cm diam., at first subglobose or hemispherical to campanulate then expanding to convex or broadly convex to finally almost applanate; surface at first whitish to grayish, then grayish to pale grayish brown and grayish brown at center, becoming yellow when rubbed, with pale gray to grayish brown fibrillose squamules, dry; margin incurved at first, then decurved, finally sometimes undulating; with remnants of partial veil attached. Lamellae free, ≤4 mm wide, at first white to pinkish, then pinkish brown to brown, finally dark brown; crowded, with lamellulae. Stipe 8–13 × 0.5–1.3 cm, central, cylindrical with bulbous base; surface white to whitish, and brownish at base, becoming yellow when rubbed, with whitish to brownish fibrils at the lower part; solid to soft, fibrous. Context white or whitish, becoming yellowish to yellow when cut, fleshy, firm. Annulus white to whitish, thin, membranous, superior. Basidiospore deposit chocolate brown to blackish brown. Taste and Odor unpleasant. Chemical reaction with 20% KOH positive.

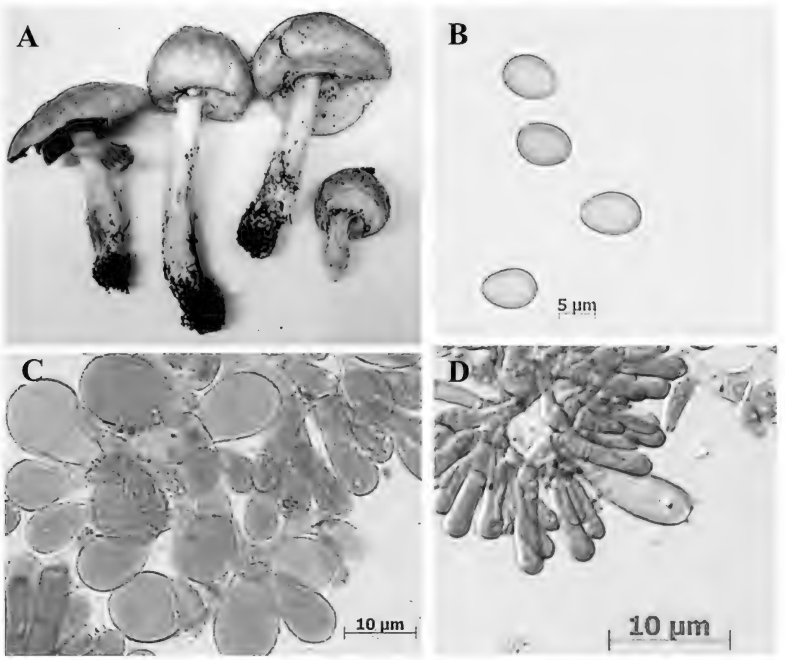

Figure 4. *Agaricus xanthodermus* (HMAS 272456):
A. basidiomata; B. basidiospores; C. cheilocystidia; D. basidia.

Basidiospores 4–5.5 × 3–4 µm, avl × avw = 4.5 × 3.5 µm, Q = 1.3–1.4, avQ = 1.35 ellipsoid, pale brown to brown, smooth, thick-walled, inamyloid. Basidia 15–25 × 6–8 µm, clavate, 4-spored, thin-walled, subhyaline to brownish. Lamella edge heterogeneous, with sterile cells, 14–22 × 4–7 µm, clavate, thin-walled, subhyaline to brownish. Cheilocystidia rare, 18–30 × 6–11 µm, pyriform, thin-walled, hyaline. Subhymenium ≤12 µm wide; hyphae narrow, branched, hyaline, thin-walled, 2.5–5 µm diam. Hymenophoral trama regular, 100–120 µm wide; hyphae hyaline, thin-walled, 4–25 µm diam. Pileipellis a radially aligned repent epicutis; hyphae thin-walled, 4.0–10 µm diam., cylindrical, branched, hyaline to subhyaline. Pileus trama hyphae 5–20 µm diam., cylindrical to inflated, hyaline, thin-walled. Annulus hyphae 4–10 µm diam., cylindrical, hyaline to subhyaline, thin-walled. Clamp connections absent.

Habitat: Growing in groups on grazing land and on soil with plant litter; in semi-open middle-aged mixed forest.

Specimens examined: **ETHIOPIA, Oromia Special Zone, Wolmera District**, Holeta, on grazing land, 10 July 2013, RSK5 (HMAS 272456; GenBank KP229414); Menagesha, Menagesha suba forest, 10 July 2013, RSK7 (HMAS 272457; GenBank KP229415).

REMARKS—*Agaricus xanthodermus* is characterized by its strong and rapid yellow coloration when cut, phenolic odor, cylindrical stipe (often with a small basal bulb), negative Schäffer reaction, and positive KOH reaction. Both macro- and micro-morphological characters observed in the Ethiopian collections were similar to those in the original description. The fungus was common and abundant on grazing land and on litter in semi-open mixed forest in the study sites starting from end of June to the beginning of August. However, ITS sequence analyses showed the clade including the Ethiopian sequences with GenBank sequences of *A. xanthodermus* and *A. moelleri* had only 83% support (FIG. 1). Comparisons of ITS sequences between our collections and *A. xanthodermus* from GenBank indicated three nucleotide differences (at positions 334, 630, and 665). More extensive analyses may reveal the true link among the sequences within the cluster.

Discussion

Morphological observation and molecular analysis placed our Ethiopian collections within two sections, *Agaricus* sect. *Agaricus* and *A.* sect. *Xanthodermatei*.

Agaricus sect. *Xanthodermatei*, represents a monophyletic clade comprising species allied to the type species of the section, *A. xanthodermus* (Kerrigan et al. 2005), known to produce one or more toxic compounds (Wood et al. 1998, Jovel et al. 1996) that can cause gastrointestinal distress leading to violent vomiting. Species in the section are generally considered inedible or not fit for human consumption. The toxicity experienced in Ethiopia after ingestion is reflected by 'Dem Astefy,' the local Amharic name for these mushrooms meaning 'causes vomiting of blood.'

In contrast, the type species of *A.* sect. *Agaricus*, *A. campestris,* is an excellent edible mushroom and abundant at the beginning of the rainy season, but the edibility of this mushroom is not recognized within the Ethiopian community. This might be due to its similarity to the toxic species in *A.* sect. *Xanthodermatei*. The confusability of the mushrooms could lead to mushroom poisoning, leading the local community to shun all the mushrooms growing in the wild and become mycophobic. This in spite of the fact that species of *A.* sect *Xanthodermatei* can usually be distinguished from similar mushrooms (like *A. campestris*) by their tendency to stain and bruise yellow and by the characteristic phenolic odor.

The high *Agaricus* species diversity of *Agaricus* underscores the need for further study of the genus; we hope that many more reports on *Agaricus* species will follow.

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Phoma candelariellae sp. nov., a lichenicolous fungus from Turkey

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Abstract — *Phoma candelariellae* is described from the apothecia of *Candelariella aurella* in the Central Anatolia region of Turkey. The new species is distinguished among the lichenicolous *Phoma* species by its clustered conidiomata arising in groups and its subglobose conidia. It is also the first *Phoma* species reported on *Candelariella*.

Key words — biodiversity, coelomycetes, mitosporic fungi, *Phaeosphaeriaceae*, *Dothideomycetes*

Introduction

Phoma Sacc. is a large fungus genus with approximately 220 species, most of which are plant pathogens, endophytes, or saprophytes (Boerema et al. 2004). Twenty-four species in this genus, however, are obligately lichenicolous (Hawksworth 1981, Hawksworth & Cole 2004, Diederich et al. 2007, Brackel 2008, Kondratyuk et al. 2010, Lawrey et al. 2012, Halıcı et al. 2014). Keys to lichenicolous *Phoma* species have been provided by Hawksworth (1981), Hawksworth & Cole (2004), and Diederich et al. (2007). Recent molecular analyses place the genus in the *Phaeosphaeriaceae*, *Pleosporales*, *Dothideomycetes* (Lawrey et al. 2012).

Halıcı (2008) published a key to the lichenicolous fungi of Turkey covering 117 taxa. Of the 190 lichenicolous species reported from Turkey (Halıcı et al. 2012, 2014), only three lichenicolous *Phoma* species have been reported: *P. peltigerae* (P. Karst.) D. Hawksw. on *Peltigera* spp. (Candan et al. 2010; Halıcı et al. 2012); *P. caloplacae* D. Hawksw. [≡ *Diederichomyces caloplacae*; Trakunyingcharoen et al. 2014] on the apothecia of *Caloplaca stillicidiorum* (Hawksworth 1981); and *P. recepii* Halıcı & Candan on the apothecia of *Caloplaca cerina* and *C. monacensis*

(Halıcı et al. 2014). A new species, described here as *P. candelariellae*, represents the fourth lichenicolous *Phoma* species from Turkey.

Material & methods

The holotype and paratype specimens of the new species are deposited in Erciyes University Herbarium Kayseri, Turkey (ERC). Sections were prepared by hand and examined in I [Lugol's iodine (Merck 9261) with (KI) and without (I) pre-treatment with 10% KOH], 10% KOH, cotton blue, and water. Conidia and pycnidia were measured in water; measurements are presented as (min–) X–sd – *X* – X+sd (–max), where 'min' and 'max' represent the extreme values, 'X' the arithmetic mean, and 'sd' the standard deviation. The microphotographs were taken with a Leica DFC 420 digital microscope camera with a c-mount interface and a 5 megapixel CCD.

Taxonomy

Phoma candelariellae Z. Kocakaya & Halıcı, **sp. nov.** Figure 1

MycoBank MB 812271

Differs from all other lichenicolous *Phoma* species by its combination of clustered conidiomata arising in groups, subglobose conidia, and host genus *Candelariella*.

Type: Turkey, Eskişehir, Sivrihisar, northwest of Sivrihisar, 39°27′16″N 31°31′36″E, alt. 1260 m, on apothecia of *Candelariella aurella* (Hoffm.) Zahlbr., 22 July 2012, M.G. Halıcı MGH3.274 (**Holotype**, EUH).

Etymology: The epithet refers to the host lichen genus *Candelariella*.

Conidiomata pycnidial, arising in groups on the apothecia of the host lichen (*Candelariella aurella*), at first more or less immersed but at maturity becoming partially erumpent, clustered, black in macroscopic view, in section pale golden brown in lower parts and dark brown in the upper parts, covered by a hyaline gelatinous sheath, subglobose to almost globose, (72–)80–*98.5*–117(–129) × (69–)74–*89*–103(–111) µm (n = 20), ostiolate, ostiole c. 12 µm diam.; pycnidial wall 9–14 µm thick, composed of 2–5 layers of pseudoparenchymatous cells, mainly polyhedral but some globose, outer cells dark brown, about 4–6 × 6–7 µm, inner ones paler, 4–7 µm wide. Cells near the ostiole have a darker brownish tinge. Conidiogenous cells lining the inner wall of the pycnidial cavity, short ampulliform to subglobose, hyaline, smooth-walled, 4–7 × 3–5 µm, conidiogenesis enteroblastic. Conidia abundantly produced, arising singly, subglobose to globose, hyaline, generally 1-guttulate, sometimes one additional small guttule present, simple, smooth-walled, with a gelatinous sheath, (3.5–)4.5–*5*–5.5(–6) × (3–)3.5–*3.9*–4.5(–5.4) µm; l/b = (0.9–)1.1–*1.3*–1.5(–1.7) (n = 100).

Additional specimen examined: **TURKEY**, **Kütahya**, Ilıca, north of Yazlıca Village, 39°36′41″N 30°03′03″E, alt. 1050 m, on apothecia of *Candelariella aurella*, 29 June 2012, M.G. Halıcı MGH3.331 (EUH).

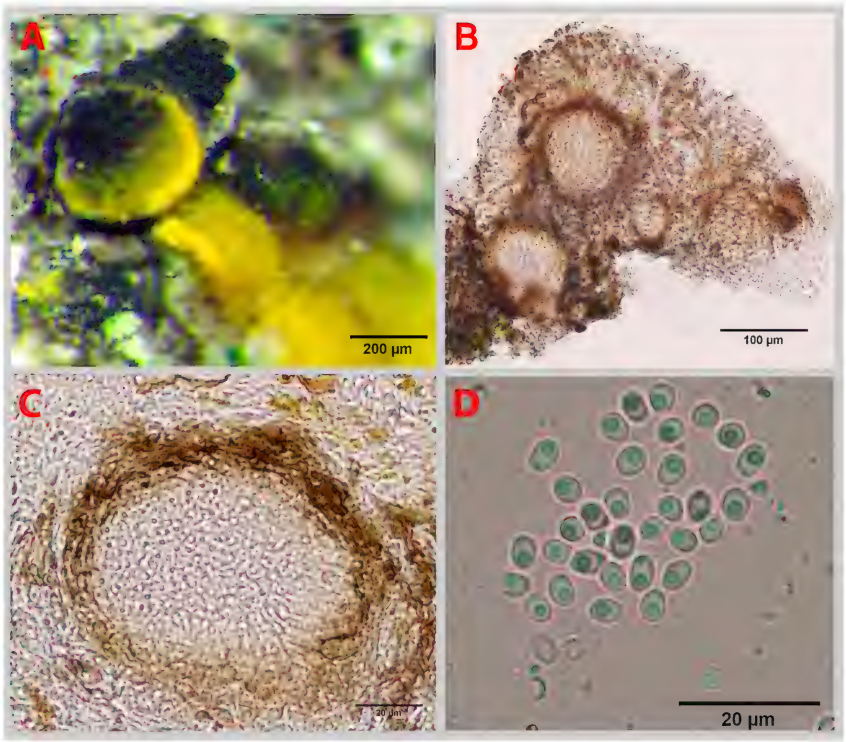

Fig. 1. *Phoma candelariellae* (holotype, ERC MGH 3.274): A, infected apothecial discs of the host lichen, *Candelariella aurella*, showing conidiomata arising in groups; B, conidiomata in the hymenium of the host; C, closer view of a conidioma; D, conidia.

Ecology & distribution: *Phoma candelariellae* is currently known from only two localities in the northwest part of the Central Anatolia Region, Turkey, on the apothecia of *Candelariella aurella* on calcareous rocks at an 1000–1300 m elevation. As the host lichen occurs on calcareous rocks throughout the northern hemisphere, *P. candelariellae* may have a potentially much wider distribution. The species seems to be pathogenic, as the infected apothecial discs of the host discolour and become blackish; ascospore production is apparently inhibited in the infected parts of the hymenium, until eventually the entire hymenium of an infected apothecium is destroyed.

Notes: *Phoma candelariellae* is the only *Phoma* species known to grow on *Candelariella*. Among the previously described lichenicolous *Phoma* species, only *P. caloplacae*, *P. lobariae* Diederich & Etayo, *P. puncteliae* Diederich

Table 1. Comparison of lichenicolous *Phoma* species with subglobose conidia

Species	Conidiomata (µm)	Conidiogenous cells (µm)	Conidia (µm)	Reference
P. caloplacae	50–130	5–6	4–7	Hawksworth (1981)
P. candelariellae	72–129 × 69–111	4–7 × 3–5	3.5–6 × 3–5.4	This paper
P. lobariae	50–100	4–6 × 1.7–3.5	3–4 × 2.5–3	Etayo & Diederich (1995)
P. physciicola	100–150	4–7	4–6 × 2.5–4	Hawksworth (1981)
P. puncteliae	40–60	2.5–5 × 3.5–4.5	2.5–3 × 2–2.4	Lawrey et al. (2012)

& Lawrey [≡ *Xenophoma puncteliae*; Trakunyingcharoen et al. 2014], and *P. physciicola* Keissl. have subglobose conidia. These species all grow on different lichen hosts and differ from *P. candelariellae* in various morphological characters (Table 1). *Phoma caloplacae* differs from *P. candelariellae* by its conidiomata arising singly in the host apothecia and its slightly larger conidia (Hawksworth 1981, Halıcı et al. 2014). *Phoma lobariae*, described on the older parts of the thallus of *Lobaria pulmonaria*, also has conidiomata arising singly and slightly smaller conidia and conidiomata (Etayo & Diederich 1995). *Phoma physciicola*, reported on *Physciaceae*, *Parmeliaceae*, and *Baeomyces rufus*, produces conidiomata that sometimes arise in small groups on the apothecia or thalli of the host lichens but is distinguished by its more ellipsoid conidia (Keissler 1911, Hawksworth 1981, Diederich et al. 2007). *Phoma puncteliae*, growing on the thalli of *Punctelia rudecta*, has obviously smaller conidia and conidiomata (Lawrey et al. 2012).

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Neoalbatrellus subcaeruleoporus sp. nov. (*Scutigeraceae*) from western North America

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Abstract –*Neoalbatrellus subcaeruleoporus* is described as a new species. Its systematic position is supported by molecular and morphological analyses, and a key to the three *Neoalbatrellus* species is provided. A revised key to American and European species of *Scutiger* sensu lato (*Albatrellopsis*, *Albatrellus*, *Laeticutis*, *Neoalbatrellus*, *Polypus*, *Polyporoletus*, *Polyporopsis*, *Scutiger* s.s., *Xanthoporus*, and *Xeroceps*) is appended.

Keywords –*Albatrellaceae*, *caeruleoporus,* correct family, genetic, *Russulales*

Introduction

The genus *Neoalbatrellus* was proposed by Audet (2010) and is distinguished from *Albatrellus* by the hymeniderm and the blue, black and brown colors of the basidiomata. Two species have been placed in *Neoalbatrellus*: *N. caeruleoporus* and *N. yasudae* (Audet 2010). Their trophic status is poorly understood and specific ectomycorrhizal host trees have not been confirmed, although *N. caeruleoporus* is often found under *Tsuga* (Gilbertson & Ryvarden 1986). The presence of extracellular laccase has been reported in basidiomata of *N. caeruleoporus* (Marr et al. 1986). The closest genus to *Neoalbatrellus* is *Albatrellopsis,* based on molecular (ITS) and the dimeric meroterpenoid pigment, grifolinone B (Zhou & Liu 2010). The correct family for these genera is *Scutigeraceae* Bondartsev & Singer ex Singer 1969, a name that has priority over *Albatrellaceae* Nuss 1980 (Audet 2010: 439).

In this paper a new species, *Neoalbatrellus subcaeruleoporus*, is described from western North America. In the past it has been confused with *N. caeruleoporus* due to similarity in basidioma color, but the two species differ both morphologically and genetically.

Table 1: Species names, vouchers, and GenBank accession numbers of the sequences used in the phylogenetic analysis.

Revised name	Deposited name	Voucher	ITS sequence
Albatrellopsis flettii	*Albatrellus flettii*	MICH AHS82164	AY621802
Neoalbatrellus caeruleoporus	*Albatrellus caeruleoporus*	MICH K.A. Harrison 8825	AY963565
Neoalbatrellus subcaeruleoporus	*Albatrellus caeruleoporus*	OSC 66097	KC985126
Neoalbatrellus subcaeruleoporus	*Albatrellus caeruleoporus*	DAVFP 28304	KC862265
Neoalbatrellus yasudae	*Albatrellus yasudae*	HKAS 357766	(Zheng 2006)

Materials & methods

This study is based on collections of *Neoalbatrellus* from western and eastern North America. The specimens were studied macroscopically, microscopically, and genetically. Color terms for basidiomata beginning with a capital letter (e.g. Cadet Gray) follow Ridgway (1912). Unless otherwise specified, the macroscopic description is based on Luther's notes from fresh material

Microscopic features were described from dried specimens (with herbarium acronyms following Thiers 2013). Basidiospores and basidia were observed using bright field microscopy at a magnification of 1250×. Melzer's reagent or IKI was used for microscopic observations of basidiospores. Basidiospores were measured in profile view with the extreme values indicated in parentheses. The number of measurements is cited as n = x/y, indicating x measurements of spores from y specimens. 100 spores were measured for all collections of *N. subcaeruleoporus* studied. The following abbreviations are used: Q = the quotient of spore length and spore width in any one spore, indicated as a range of variation in n spores measured; L_m = mean spore length (µm), W_m = mean spore width (µm), Q_m = quotient of mean spore length and mean spore width (L_m/W_m ratio).

Hyphae, basidia, and spores were observed in cotton blue in lactic acid to verify the presence of gloeoplerous hyphae and cyanophilous (CB+) elements. The cyanophilous thin-walled hyphae appear blue in mass.

The protocols for DNA extraction and amplification of the ITS1+5.8S+ITS2 region in *N. subcaeruleoporus* were done by R.S. Winder and M. Gordon, who provided two *subcaeruleoporus* sequences. Winder slightly modified the method of Kranabetter et al. (2013) by not soaking the sample overnight but by freezing a 50 mg portion of the sample in liquid N and grinding in a sterile mortar and pestle before adding the CTAB buffer. For PCR amplification, the universal primer pair ITS5/ITS4 was used (White et al. 1990; Gardes & Bruns 1993); the PCR product was sequenced by the Genome Sequencing and Genotyping Platform (CHUL Medical Research Centre (Quebec, Canada) using capillary electrophoresis on an ABI 3730/XL Analyzer (Applied Biosystems). Gordon extracted DNA from 50 mg of dried mushroom using the UltraClean DNA Isolation

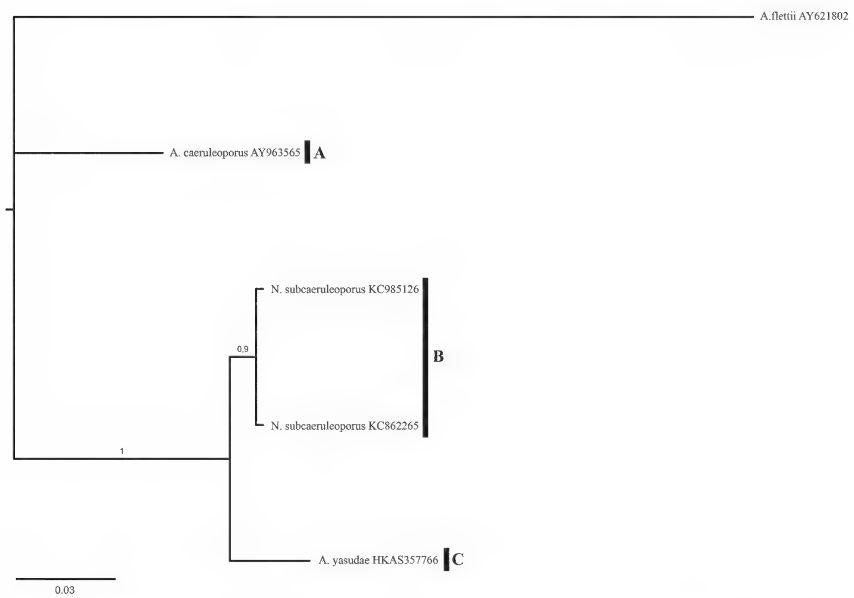

FIGURE 1: rDNA ITS1+5.8S+ITS2 strict consensus tree as generated using MrBayes (Huelsenbeck & Ronquist 2001; 1,000,000 generations). A: *Neoalbatrellus caeruleoporus*; B: *Neoalbatrellus subcaeruleoporus*; C: *Neoalbatrellus yasudae*; outgroup: *Albatrellopsis flettii.*

Kit (MoBio Laboratories, Inc.) and amplified the ITS region following a standard PCR protocol using primers ITS1f and ITS4 with an anneal temperature of 58°C. The ITS amplicon was sequenced at Eurofins MWG Operon (operon.com).

The sequences used in the phylogenetic analysis are presented in TABLE 1. *Albatrellus flettii* [≡ *Albatrellopsis flettii*] served as outgroup.

Taxonomy

The phylogeny inferred from the ribosomal ITS1-5.8S-ITS2 sequences from three samples labeled *Albatrellus caeruleoporus* and one each of *A. flettii* and *A. yasudae* is shown in FIG. 1. One *A. caeruleoporus* sequence (AY963565) from eastern North America formed a separate clade (A). Two *caeruleoporus* sequences (KC985126, KC862265) from British Columbia and California formed a distinct and strongly supported clade (B). The sequence labeled *A. yasudae* (HKAS357766) from China formed a third clade (C) separated from the two *A. caeruleoporus* clades. The samples in clade B are considered as representing an independent taxon, described here as *Neoalbatrellus subcaeruleoporus*. We obtained nearly a complete ribosomal ITS sequence from our *N. subcaeruleoporus* holotype and alignment supports it as a new species.

Neoalbatrellus subcaeruleoporus Audet & B.S. Luther, **sp. nov.** FIGS 2A, 3

MYCOBANK MB801480

Differs from *Neoalbatrellus caeruleoporus* by its paler blue fresh basidiomata, its gloeoplerous hyphae, its smaller basidia and basidiospores, and its western North American distribution.

TYPE: USA. Washington state, Yakima Co., Bumping Lake Road, in William O. Douglas Wilderness across the footbridge from Soda Springs Campground, a.s.l. 918 m, in conifer duff and forest litter directly under *Alnus* sp., with *Abies grandis* and *Pseudotsuga menziesii* nearby, 7 October 1995, coll. B.S. Luther 1995-107-1, det. S. Audet (**Holotype**, WTU-F-50696).

ETYMOLOGY: from the bluish colors shared with *Neoalbatrellus caeruleoporus*.

PILEUS 3–6.5 cm wide × 3–5.5 mm thick, umbonate to convex, irregularly plane or more frequently depressed, orbicular, flabelliform, dimidiate or reniform, firm when fresh, drying hard and brown, black laccate, or greenish–brown and sometimes resinous reddish in areas; surface irregular, dry, glabrous to finely rugose or subtomentose; color a Pale Glaucous Blue at first, later Cadet Gray to Light Mineral Gray with margin often paling to Pale Smoke Gray to Light Olive Gray, when drying reddish undertones developing with ochraceous–orange or orangish areas showing in pileal cracks or where damaged (rodent bites, etc.); MARGIN uniform, convoluted or irregular and inrolled, with lobes or undulations and splits (clefts), concolorous to generally much paler than the pileus, with orangish or ochraceous tints in age, surface glabrous and soft or breaking up into minute scales that are darker than the underlying tissue and slightly roughened, with an appendiculate, sterile edge, when mature prominently incurved or slightly involute and somewhat even; CONTEXT ≤1 cm thick, white at first, slowly Pale Ochraceous Buff along margins, then

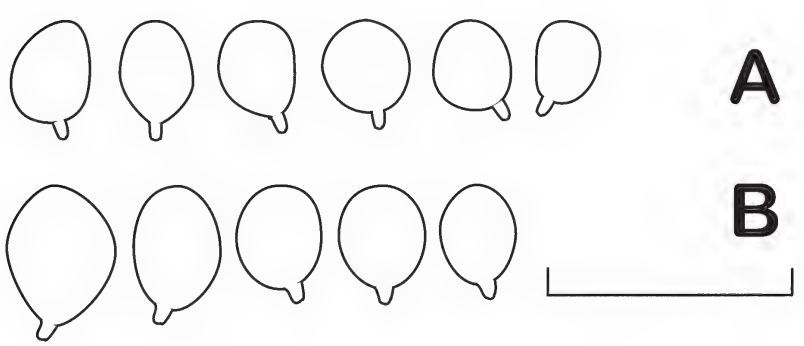

FIGURE 2. Basidiospores (Scale bar = 9.2 µm).
A. *Neoalbatrellus subcaeruleoporus* (holotype). B. *Neoalbatrellus caeruleoporus* (isotype).

FIGURE 3. *Neoalbatrellus subcaeruleoporus* (WTU-F-50695).
Fresh basiodiomes (one cut to show context).

Light Pinkish Cinnamon or Light Vinaceous Cinnamon; when dried cinnabar necropigments light, becoming darker after extended storage; ODOR sweetly fungoid or not distinctive; TASTE mild and slightly sweetish to slightly peppery. PORES 1–3 per mm (3–6 per mm in dried OSC 66097), orbicular, elongate to slightly angular, pruinose inside, with orange–pinkish necropigments; near the stipe uniform, ≤1 mm wide, concolorous with or paler than the pileus, smaller at the growing margin, uniformly pale bluish and with pale ochraceous or pale orangish to rusty areas at maturity or when injured/bruised, very pale when dry. TUBES decurrent, shallow, 1–2 mm deep, dissepiment walls paler (very pale when dry), fimbriate or granulose. STIPE 3–6 × 1–2 cm, central to strongly eccentric, tapering noticeably toward the base and widening/enlarging toward the pileus, somewhat irregular to slightly twisted, curved or bent, surface smooth to innately fibrillose streaked or with very fine scaly patches, generally sub-cylindric or slightly flattened, fasciculate; surface color concolorous with the pileus, paler and often with very slight pale pinkish overcast tones, or becoming slightly darker or grayer toward the extreme base or below, but paler and almost whitish near the base where starting to taper and beneath where forest duff has adhered to the base, in age with orangish or ochraceous tints; when dried resinous reddish-brown in some areas; context solid in cross-section, pallid when freshly cut, turning pale pinkish very slowly.

Hyphal system monomitic. Pileipellis hymeniderm, hyphae in a tight palisade covered with a brownish resin, narrowly clavate with ends sometimes inflated to 7.1 µm diam., refractive, hyaline to yellowish in IKI, inamyloid with weakly cyanophilous walls. Stipitipellis hyphae similar. Contextual hyphae 2.8–11.3 µm diam. (larger hyphae often ramified and with variable diameters), simple-septate, hyaline, thin-walled, inamyloid, walls weakly cyanophilous; gloeoplerous hyphae occasional, ramified and flexuous, refractive and pale yellow in IKI, intense blue in cotton blue (CB+, cyanophilous). Tramal hyphae 2.8–3.5 µm diam., simple-septate, hyaline, thin-walled, inamyloid, walls weakly cyanophilous, often flexuous and ramified, sometimes with perpendicular branching before or near septa; gloeoplerous hyphae refractive and yellow in IKI, intense blue (cyanophilous) in cotton blue. Basidia 25–33 × 6–7 µm, narrowly clavate, wall weakly cyanophilous, contents blue with yellowish brown inclusions in cotton blue; sterigmata four, straight or slightly curved, ≤4.8 µm long. Basidiospores amygdaliform, ellipsoid to subglobose, hyaline, thin-walled, smooth, inamyloid, weakly to moderately cyanophilous, sometimes with a large brownish-yellow guttule in cotton blue, with a relatively long apiculus, (3.3–)3.6–4.7(–5.1) × (2.8–)2.9–3.7(–3.9) µm, n = 100/4; L_m × W_m = 4 × 3.2 µm; Q = 1.1–1.4(–1.5); Q_m = 1.3; half of the spores with a flat to slightly convex adaxial side.

Macrochemical reactions on context of fresh pileus stipe context: KOH (3%): Pale Pinkish Cinnamon to Light Pinkish Cinnamon, darkening the longer it sits in the reagent. NH_4OH (30%): very pale or similar to KOH reactivity. Ferric sulfate: a distinctive reddish-brown exudate that completely darkens the solution around the fresh sample. Tincture of guaiac resin: –. Phenol: –. Aniline: –. PDAB(4-dimethyl amino benzaldehyde): + (striking bright turquoise after 5 minutes).

Additional specimens examined (all as ***Albatrellus caeruleoporus***): **U.S.A. Washington State: King Co.**, Lake Quinault, Rain Forest Mushroom Festival, 18 October 2008, Steve Trudell (WTU-F-13070); **Pierce Co.**, Mt. Rainier National Park, Carbon River area, 15 October 1987, B.S. Luther 1987-1015-1 (WTU-F-50684); **Skamania Co.**, Gifford Pinchot National Forest, Curly Creek Falls Trailhead, 5 October 2008, W. Foster SKAWA001 (WTU-F-13071); **Yakima Co.**, Bumping Lake Road, William O. Douglas Wilderness from Soda Springs Campground, 1 October 1994, B.S. Luther 1994-101-1 (WTU-F-50695); 23 September 1995, B.S. Luther 1995-923-1 (WTU-F-50681). **Oregon: Lane Co.**, JM Honeyman Park, 12 November 1992, George Barron (DAOM 215968); **California: Humboldt Co.**, Redwood National Park ("Prairie Creek"), UTM N: 415268, UTM E: 4582445 zone 10, 12 December 1997, T. O'Dell 4550 & M. Madsen (OSC 66097). **CANADA. British Columbia: (Mainland)** N of Clearwater, near Spahats, 10 September 1982, T. Goward (DAOM 194800); Mt. Elphinstone, Roberts Creek, 29 September 2000, Paul Kroeger (DAOM 229493); **Vancouver Island,** just west of Sooke, close to Juan de Fuca Strait, second growth, 28

October 2010, P. Hutchinson & K. Trim (DAVFP 28293); Sooke, Otter Point Road, 22 November 2011, K. Trim (DAVFP 28304).

Ecology & Distribution— Arising from deep in forest duff during mid-September to mid-December. Associated with *Abies grandis*, *Pseudotsuga menziesii*, *Alnus* sp., *Picea sitchensis, Sequoia sempervirens, Tsuga heterophylla*. It fruited in an arc around the base of *Alnus* sp. but conifers were also close by and mixed in. Rare in western North America in northwestern California, central and southwestern British Columbia, western Oregon, and northwestern and central Washington on the east slopes of the Cascades.

Other specimens examined: ***Neoalbatrellus caeruleoporus:*** **CANADA. Québec:** Quebec, 1999, det. R. Labbé (QFB-8563); Richmond County, Kingsbury, 1984, det. R. Cauchon (QFB-16558L); Beauce, Sainte-Clothilde, late August 2011, coll. M. Nadeau, det. R. Labbé (WTU-F-50683); **Nova Scotia:** Annapolis Co., Bishop Mt. Road, 19 October 1969, coll. & det. K.A. Harrison (MICH 00073662; KAH 8825). **U.S.A. New York State:** near Oneonta, 1963, det. S. Smith (QFB-199L,); Duchess County, Copake, October, leg. & det. C.H. Peck (NY 00730549, isotype of *Polyporus caeruleoporus*).

Remarks: *Neoalbatrellus subcaeruleoporus* is considered rare and has been listed (as *Albatrellus caeruleoporus*) for protection and received special conservation status in the United States (Molina 2008). Also as *A. caeruleoporus,* it has been cited as a possible indicator of western old growth forests and considered in forest management plans (Ginns 1997).

A detailed comparison of *Neoalbatrellus subcaeruleoporus* and *N. caeruleoporus* is presented in Table 2.

The pores of *N. subcaeruleoporus* are somewhat smaller and orbicular to angular with a thicker wall than those of *N. caeruleoporus*. Our *N. subcaeruleoporus* basidiospore measurements are slightly smaller than those given by Ginns (1997, as *A. caeruleoporus*). The dull colors (bluish-gray, with a tinge of orange) not seen in the very young, actively growing basidiomata and appearing only in fully mature specimens, are typical of basidiomata collected late in the season.

It is interesting to note that *N. yasudae* is characterized by basidiospore sizes intermediate (4–5.5 × 3.5–5 µm; Zheng & Liu 2008) between *N. subcaeruleoporus* (3.6–4.7 × 2.9–3.7 µm; Table 2) and *N. caeruleoporus* (4.6–5.6 × 3.8–4.3 µm; Table 2) but shares with *N. subcaeruleoporus* a pileipellis covered with a brownish resinous substance (as seen under the microscope) and a pale hymenophore. These morphological distinctions are supported by ITS sequence analysis.

Concerning macrochemical reactions, ferric sulfate in solution is not as reactive as ferrous sulfate. The strong reaction of the *N. subcaeruleoporus* context in ferric sulfate suggests that ferrous sulfate will likely have a stronger reaction

Table 2: Comparison between *Neoalbatrellus subcaeruleoporus* and *N. caeruleoporus*

Character	*N. subcaeruleoporus*	*N. caeruleoporus*
Cap, dry	Becoming brown or brown to black, with tubes and context over time turning very slowly cinnabar orange	Becoming brownish, with the entire basidiome eventually often turning cinnabar orange
Cap, fresh	≤6 cm in diameter	≤15 cm in diameter [1]
	Light blue to pale blue, often glaucous with reddish orange-reas where damaged, with age, or in cracks	Grayish blue to violaceous, in age tan to light ochraceous brown [2]
Context, in herbarium	White to pale orange (when exposed)	Typically pale orange (also pallid or orange pink) [2]
Context, fresh	White to pale wood brown; slowly discoloring after a few hours in open air to pale ochraceous buff, light pinkish cinnamon, or light vinaceous cinnamon	White [2]
Tubes, fresh	0.7–2 mm long	2–5 mm long [1]
	Concolorous with the cap or paler, becoming orangish or rusty irregularly or where damaged	Grayish blue [2]
Stipe, fresh	Without lateral stipe	With lateral stipe when young
	Concolorous with the pileus or paler; context bruising orange-brown	Deep indigo-blue [1]
KOH on context	Pale or light pinkish cinnamon, darkening over time (3%)	Not discoloring (2%) [2]
30% NH_4OH on context	Very pale or as above (KOH)	Not tested
Phenol on context	Negative	Not tested
Analine on context	Negative	Not tested
Ferric sulfate ($Fe_2(SO_4)_3$)	Context reddish-brown color	Not tested
Ferrous sulfate ($FeSO_4$)	Not tested	Context staining pale yellow [2]
Tincture of guaiac resin (on context)	Negative	Slowly positive blue reaction (≥30 minutes) when tincture replenished
Syringaldazine (laccase test)	Not tested	Reddish (positive: 0.5/4) [4]
Gloeoplerous hyphae	Scattered in context (trama) [3]	Not seen [2]
Basidia	23–32(–44) × 5.6–7.4 µm [3]	33–48 × 6–8 µm [2]
Basidiospore apiculus	Relatively long, tapered [3]	Small, but obvious [2]
Basidiospores	4.0–4.8(–5) × 3.4–3.6(–4) µm [3]	4.8–5.6 × 3.8–4.2(–4.8) µm [2]
	3.6–4.7 × 2.9–3.7 µm, n = 100/4	4.6–5.6 × 3.8–4.3 µm, n = 40/2
	(DAVFP 28293; OSC 66097; WTU-F-50696; WTU-F-50684)	(NY 00730549; MICH 00073662)

[1] Overholts (1953); [2] Ginns (1994); [3] Ginns (1997, as *Albatrellus caeruleoporus*); [4] Marr et al. (1986).

on *N. subcaeruleoporus* than on *N. caeruleoporus*. It is interesting to note that *N. caeruleoporus* gives a slow positive reaction with an alcoholic solution of guaiac resin, but *N. subcaeruleoporus* does not. The positive reaction with this reagent simply shows the presence of extracellular phenol-oxidases (tyrosinase, laccase, and peroxidase) and is not specific for a particular enzyme (Gilbertson et al. 1975). This reaction is consistent with a study by Marr et al. (1986) who show a positive laccase reaction with syringaldazine for *N. caeruleoporus*.

Key to species of *Neoalbatrellus*

1. Pileipellis with densely agglutinated pyriform cells ..... ***N. yasudae***
   Pileipellis without densely agglutinated pyriform cells 2
2. Basidioma pale blue; western North American distribution;
   spores 3.6–4.7 × 2.9–3.7 µm ..... ***N. subcaeruleoporus***
   Basidioma blue; eastern North American distribution;
   spores 4.6–5.6 × 3.8–4.3 µm ..... ***N. caeruleoporus***

Summary

The genus *Neoalbatrellus* has an additional species, *N. subcaeruleoporus*. It is separated from *N. caeruleoporus* by its pale blue basidioma, shorter basidia and basidiospores, lack of a phenol-oxidase reaction, and a western North American distribution, as well as by its DNA sequences.

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Appendix 1. Key to American and European species of *Scutiger* s. lat.
(From Audet 2010; revised by Serge Audet & Georges Fannechere)

1. Clamp connections on contextual generative hyphae numerous 2
   Clamp connections on contextual generative hyphae absent or very rare 15
2. Spores ≤12(–14) µm long 3
   Spores smaller or equal to 7 µm long 10
3. Spores without double wall 4
   Spores with double wall 7
4. Spores not primarily tear-drop shaped 5
   Spores primarily tear-drop shaped 6
5. Pileus brown (fresh) or dark violet (dried) ..... ***Xeroceps skamania***
   Pileus yellow (fresh) or pinkish white to pale red (dried) .... ***Xeroceps yunnanensis***
6. Pileus greenish olive to milk chocolate brown ..... ***Scutiger ellisii***
   Pileus dark brown ..... ***Scutiger pes-caprae***
7. Hyphal system dimitic ..... ***Polyporopsis mexicana***
   Hyphal system monomitic 8
8. Spores c. 9.6–12.6 µm diam. ..... ***Polyporoletus sublividus* s. lat.**
   Spores c. 7.2–10.8 µm diam. 9
9. Basidiomes solitary and with bulbous stipe ..... ***Polyporoletus bulbosus***
   Basidiomes caespitose and with no bulbous stipe ..... ***Polyporoletus sylvestris***
10. Hymenophore entirely yellow when young 11
    Hymenophore not entirely yellow when young 12
11. Some thick-walled hyphae (often >0.5 µm) near the surface from stipe base; spores small (3.5–4.5 × 2.5–3 µm); pores small (4–6 per mm) ..... ***Xanthoporus peckianus***
    No thick-walled hyphae near the surface from stipe base; spores larger (4–5.6 × 3–4 µm); pores larger (3–5 per mm) ..... ***Xanthoporus syringae***
12. Spores elongated to cylindrical, 4.8–6.4 × 2–2.5 µm ..... ***Albatrellus pilosus***
    Spores not cylindrical 13
13. Spinose hyphae present on the mycelium and covering the stipe base; spores weakly amyloid 14
    No spinose hyphae; spores amyloid or not 15
14. Pileus variably colored when fresh (chamois, apricot, rarely brownish) but not blue ..... ***Albatrellopsis confluens***
    Pileus partly or entirely blue when fresh ..... ***Albatrellopsis flettii***

15. Pileus surface laccate ..... ***Albatrellus arizonicus***
    Pileus surface non-laccate 16
16. Pileus with black squamules and resinous dark zone between tubes and context ..... ***Albatrellus tianschanicus***
    Pileus without black squamules 17
17. Basidiome with numerous petaloid pilei from common base; pileus golden when fresh ..... ***Polypus dispansus***
    Basidiome with solitary pileus or confluent 18
18. Majority of spores longer than 5.5 µm ..... ***Laeticutis cristata***
    Majority of spores shorter than 5.5 µm 19
19. With hymeniderm; basidiome blue when fresh 20
    Without hymeniderm; basidiome not blue when fresh 21
20. Occurring in western North America; spores 3.6–4.7 × 2.9–3.7 µm ..... ***Neoalbatrellus subcaeruleoporus***
    Occurring in eastern North America; spores 4.6–5.6 × 3.8–4.3 µm ..... ***Neoalbatrellus caeruleoporus***
21. Spores inamyloid; associated with *Tsuga*, *Picea*, or *Abies* 22
    Spores distinctly amyloid; associated with especially *Pinus* or *Picea* 23
22. Spores 5–5.6 × 3.6–4.2 µm; under conifers, especially *Tsuga* and *Picea;* on west coast of North America..... ***Albatrellus avellaneus***
    Spores 4.0–4.8 × 3.2–3.8 µm; under conifers, especially *Picea* and *Abies;* widely distributed..... ***Albatrellus ovinus***
23. Under *Pinus;* basidiome yellow or orange where bruised or with age; taste bitter or mild ..... ***Albatrellus subrubescens***
    Under *Picea;* basidiome yellow where bruised; taste mild .... ***Albatrellus citrinus***

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New *Acrocordia* and *Candelariella* records for Turkey

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Abstract — *Acrocordia subglobosa*, *Candelariella faginea*, and *C. subdeflexa* are reported for the first time from Turkey. Comments on habitats, substrata, and key anatomical features are provided for each species.

Key words — *Ascomycota*, lichens, lichenized fungi, biodiversity

Introduction

Turkish lichenology has a relatively short history compared to Europe and America. In the last 30 years, however, numerous papers have been published, including checklists of several regions in Turkey (e.g., John 2007, Halıcı & Aksoy 2009, Kınalıoğlu 2010a, Şenkardeşler 2011, Yazıcı et al. 2013, Kocakaya et al. 2014). About 1300 species of lichenized fungi are known from Turkey, but unfortunately there is no checklist of the whole country. At least 2000 lichenized fungi are expected to occur in the country, considering its size, phytogeographical diversity, and different habitats (Halıcı et al. 2007). It is clear that the lichen biota is still far from being completely known in Turkey and that taxonomic studies focusing on genera or species complexes are needed.

The genus *Candelariella* includes species with 8- or polyspored asci of the *Lecanora*-type, biatorine or lecanorine apothecia, and a secondary chemistry with pulvinic acid and derivatives (Westberg 2005). Approximately 50 *Candelariella* species are known worldwide (Westberg 2004), and in Turkey 15 species—*C. aggregata* M. Westb., *C. antennaria* Räsänen, *C. athallina* (Wedd.) Du Rietz, *C. aurella* (Hoffm.) Zahlbr., *C. coralliza* (Nyl.) H. Magn., *C. kuusamoensis* Räsänen, *C. lutella* (Vain.) Räsänen, *C. medians* (Nyl.) A.L. Sm., *C. plumbea* Poelt & Vězda, *C. reflexa* (Nyl.) Lettau, *C. rosulans* (Müll. Arg.)

Zahlbr., *C. unilocularis* (Elenkin) Nimis, *C. viae-lacteae* G. Thor & V. Wirth, *C. vitellina* (Hoffm.) Müll. Arg., *C. xanthostigma* (Pers. ex Ach.) Lettau—have been reported (Breuss & John 2004; Candan & Türk 2008; Güvenç et al. 2006; Halıcı & Aksoy 2009; Halıcı & Güvenç 2008; Halıcı et al. 2013, 2014; Yazıcı et al. 2012). Here we report two new *Candelariella* species for Turkey.

The genus *Acrocordia* is characterized by cylindrical asci with an apparently unique apical structure and by uniseriate ellipsoid ascospores with a warted perispore (Smith et al. 2009). Five species—*Acrocordia cavata* (Ach.) R.C. Harris, *A. conoidea* (Fr.) Körb., *A. gemmata* (Ach.) A. Massal., *A. macrospora* A. Massal., *A. salweyi* (Nyl.) A.L. Sm.)—have been recorded in Turkey (Doğru & Güvenç 2007, Güvenç et al. 2006, Kınalıoğlu 2010b, Özdemir Türk & Güner 1998, Yazıcı & Aslan 2006, Yazıcı et al. 2007). Here we report one new *Acrocordia* species for Turkey.

Material & methods

The newly recorded specimens are deposited in Erciyes University Herbarium Kayseri, Turkey (EUH). They were examined by standard microscopic techniques. Hand-cut sections were studied in water, potassium hydroxide (KOH), and Lugol's solution (I). Measurements were made in water. Ascospores were measured from five different ascoma for each species. The measurements are given as minimum–maximum from N measurements. The descriptions summarized below for each species are based the Turkish specimens.

Taxonomy

Acrocordia subglobosa (Vězda) Poelt & Vězda, Biblioth. Lichenol. 9: 11 (1977)

FIGS 1A,B

A detailed description is provided by Poelt & Vězda (1977).

Thallus immersed to poorly developed, whitish. Perithecia 0.3–0.7 mm diam. (N = 10); involucrellum incurved around the exciple and continuous below, black, globose. Asci cylindrical, 120–156 × 10–14 µm. (N = 15), 8-spored, ascospores uniseriately arranged in the asci. Hamathecium of pseudoparaphyses that are not appreciably widened at the tips, 1.2–1.4 µm diam. Ascospores colourless, simple, 1-septate and with a warted perispore, broadly ellipsoid, 12–18 × 7–8.5 µm (N = 20).

SPECIMEN EXAMINED: **TURKEY**, **ZONGULDAK**, Kozlu, North of Eregli–Akcakoca highway, 41°25′26″N 31°43′11″E, alt. 0–10 m, coastal rocks, 27 July 2012, M.G. Halıcı (ERC MGH 4652).

The specimen was collected from coastal calcareous rocks in Turkey's western Black Sea Region; its ascospores are slightly longer and wider than those cited in the literature (Poelt & Vězda 1977, Smith et al. 2009, Pykälä 2008).

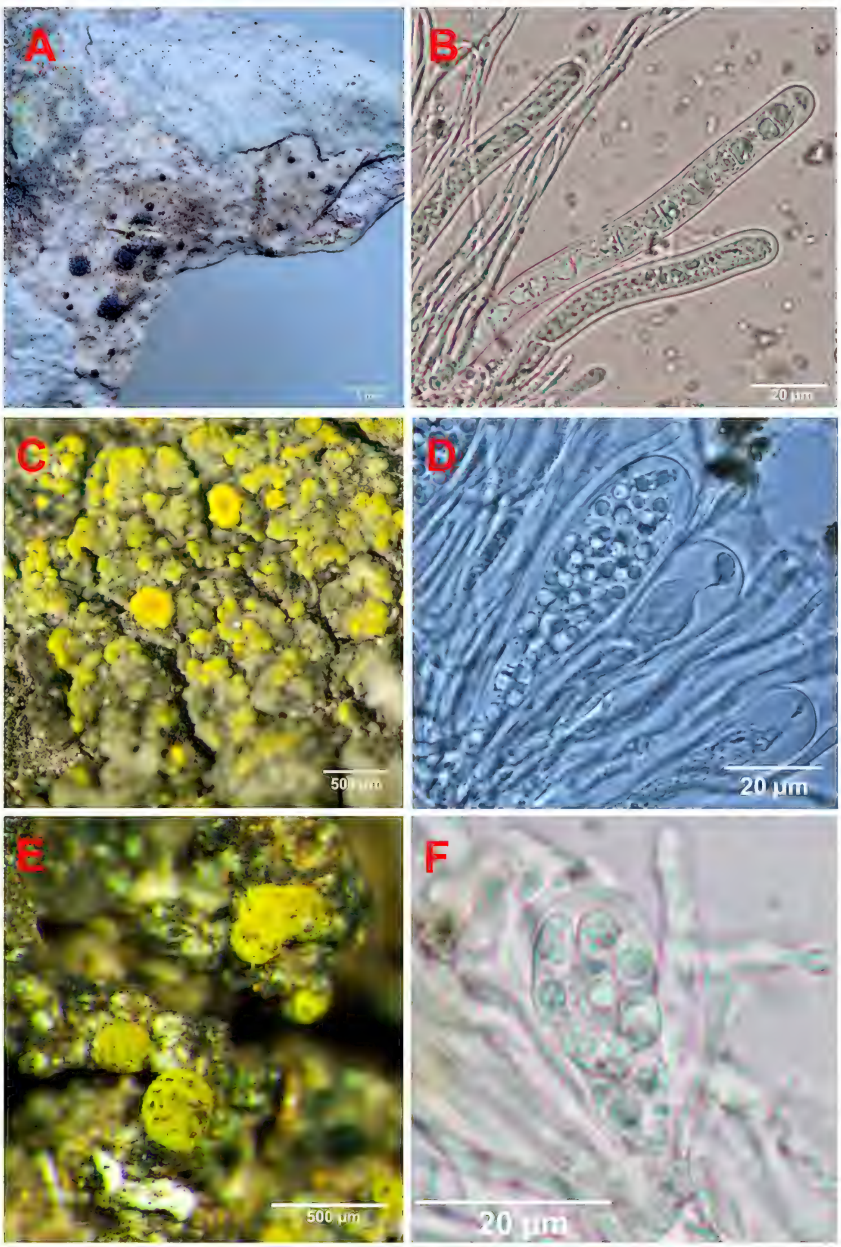

FIG. 1. ***Acrocordia subglobosa:*** A, thallus; B, ascus, ascospores, and pseudoparaphyses. ***Candelariella faginea:*** C, thallus; D, paraphyses and polyspored asci. ***Candelariella subdeflexa:*** E, thallus; F, paraphyses and 8-spored asci.

Acrocordia subglobosa is very similar to *A. salweyi*, differing mainly in its smaller perithecia and ascospores (Smith et al. 2009), and is known on calcareous rocks at 500 m from Scotland, Central Europe, Finland, and Ukraine (Smerechynska 2005, Pykälä 2008, Smith et al. 2009). New to Turkey.

Candelariella faginea Nimis, Poelt & Puntillo, Nova Hedwigia 49: 276 (1989) FIGS 1C,D

A detailed description is provided by Nimis et al. (1989).

Thallus areolate to subsquamulose; areoles grayish green in the center but yellowish green towards the margins; with blastidia. Apothecia common, lecanorine, disc greenish yellow and margin yellow, 0.2–0.4 mm diam. (N = 8). Hymenium colorless, 70–80 µm tall. Paraphyses simple. Asci clavate, polyspored, 65–80 × 20–29 µm (N = 10). Ascospores simple, narrowly ellipsoid, 13–16 × 4–6 µm (N = 15). Spot tests K–, KC–, C–.

SPECIMEN EXAMINED: **TURKEY**, **İZMIR**, Bornova, northeast of Sancaklı, 38°31′13″N 27°11′35″E, alt. 700 m, on bark of *Quercus* sp., 30 June 2012, M.G. Halıcı (ERC CAN 0. 212).

Candelariella faginea has hitherto been reported on bark of deciduous trees (usually *Fagus*). The Turkish specimen was collected on *Quercus* bark in the Aegean part of Turkey where a Mediterranean climate prevails. It was accompanied by *Caloplaca monacensis* (Leder.) Lettau, *Lecidella elaeochroma* (Ach.) M. Choisy, *Lecanora hagenii* (Ach.) Ach., *Physconia* sp., and *Rinodina* sp.

Candelariella faginea differs from *C. xanthostigma* by its blastidiate thallus and areoles that are almost squamulose. *Candelariella coralliza* differs from *C. faginea* by its thallus composed of coralloid cylindrical granules forming compact cushions and also by substrate and general ecology (Nimis et al. 1989).

This species is mainly known from southern Europe (Italy, Spain, Greece) and Ukraine (Aragon & Martinez 2002, Khodosovtsev 2005, Spribille et al. 2006). New to Turkey.

Candelariella subdeflexa (Nyl.) Lettau, Hedwigia 52: 196 (1912) FIGS 1E,F

A detailed description is provided by Westberg (2007).

Thallus squamulose, distinct, surface dirty gray, smooth. Apothecia biatorine, scattered, pale yellow to greenish yellow, 0.3–0.5 mm diam. (N = 10). Hymenium colorless, 50–60 µm tall. Paraphyses simple. Asci clavate, 8-spored, 38–45 × 13–19 µm (N = 15). Ascospores simple, narrowly ellipsoid, 9–4 × 4–5.5 µm (N = 20). Spot tests K–, KC–, C–.

SPECIMEN EXAMINED: **TURKEY**, **SIVAS**, Gemerek, Sızır, 39°18'30"N 35°59' 25"E, alt. 1250 m, on bark of *Quercus* sp., 3 October 2010, M.G. Halıcı & M. Kocakaya (ERC CAN 0.049).

The Turkish specimen was collected from Central Anatolia on the bark of *Quercus*. According to Westberg (2007), *C. subdeflexa* is a corticolous species growing mainly on the trunks of various deciduous trees such as *Populus*, *Quercus*, *Fraxinus*, and *Juniperus* and is characterized by its biatorine apothecia and gray squamulose thallus. The Turkish specimen agrees well with the description given in Westberg (2007).

This species has a bipolar distribution and is known from North America, Europe, North Africa, and New Zealand (Poelt & Vězda 1977; Nimis 1993; Galloway & Hafellner 2002; Westberg 2004, 2007; Westberg & Clerc 2012). New to Turkey.

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Book reviews and notices*

Abstract—Books reviewed include Distribution, ecology & status of 51 macromycetes in Europe (Fraiture & Otto 2015).

Fungal conservation

Distribution, ecology & status of 51 macromycetes in Europe. Results of the ECCF Mapping Programme. By A. Fraiture & P. Otto (eds), 2015. Scripta Botanica Belgica 53. <http://shopbotanicgarden.weezbe.com> ISBN 978-9-082-35255-9; 247 p, numerous maps and illustrations. Price € 25.00.

One of the main obstacles for fungal conservation is knowledge about distribution, ecology, life history, and changes in occurrence over an entire area. This is particularly well understood by the European Council for the Conservation of Fungi, which has worked for the last decade and a half on gathering that type of information for 50 species of easily recognizable basidiomycetes and ascomycetes that have been considered threatened in several countries in Europe, representing different vegetation types and distribution patterns. The final list covers 51 species, because it became clear that *Poronia punctata* records represented two species, *P. punctata* and *P. erici*, and both are included in the final list. Another criterion was that at least two of those 50 species should occur in each country.

National coordinators gathered the data concerning these species, and the two editors, with help from mapmakers and others, compiled all data (including those from the literature) in the present publication. The maps use a UTM grid on which the dots represent areas up to 2500 km^2.

The introduction gives an overview of the various problems that must be overcome in such a collaborative project concerning the occurrence of

* Book reviews or books for consideration for coverage in this column should be sent to the *Editor-in-Chief* at editor@mycotaxon.com or 6720 NW Skyline, Portland OR 97229 USA.

ephemeral basidiomes, discusses distribution patterns among species linked to vegetation, climate, and host trees, and provides a list of the contributing countries and their mycological coordinators.

The main part of the book gives the information about the species. For each species, the accepted name, notes on nomenclature, an abstract, geography, nutritional mode and host, habitat, phenology and frequency, and threats and conservation status are given. Also presented are photos of the fungi and a distribution map comparing data before and after 1970, and in some cases a map of the host tree.

In other words, a wealth of information is presented so that distribution patterns independent of the inventory efforts emerge, and generalizations can be made beyond the separation of northern and southern species.

Changes in distribution are not easily inferred from the maps, but a few species, especially some hydnaceous fungi, do show a distinct decrease in records over at least part of their area. Decline is also very apparent for *Poronia punctata*, although it maintains a stronghold in those countries where horses are used for nature management purposes.

This is not the first publication on macromycete distribution in Europe; Lange (1974) compiled data during an earlier similar initiative that did not focus on threatened fungi but was similarly aimed at producing information on fungi throughout Europe. Her publication also covered 50 species, ten of which are included in the 2015 work. Increase in mycological activities in the Mediterranean countries since 1974 is very obvious in maps of *Amanita caesarea* and *Panaeolus (Annellaria) semiovatus*.

Of course, there are mistakes and things that could have been done better. For instance, I miss a map showing the coverage of national mapping and recording schemes (before and after 1970) that would be particularly insightful for indicating distribution patterns within Russia. There are several small mistakes in the nomenclature and references (e.g., Berkeley and Brome instead of Berkeley and Broome; omission of the combination authority for *Craterellus melanoxeros;* and misspelling of an author's name, Nieves-Rivera). The text would have been easier to read if a native English speaker had reviewed it. Some species represent a group of species – this has been recognized for *Pisolithus arrhizus*, but it is also the case for *Battarrea phalloides* (Martín et al. 2013), and *Sarcosphaera coronaria* (compare nrITS data in Genbank). And, the occurrence of European species in North America and elsewhere outside Europe should be critically approached. The western North American "*Entoloma bloxamii,*" which has been shown to differ from the European species, has recently been described as a separate species, *E. medianocte* (Morgado et al. 2013; Schwarz

2015), while the ITS sequences of *Suillus umbonatus* from western North America are identical to those of European *S. flavidus*.

However, these are all small comments on a project that has to be fully applauded. We hope the project will be followed up in Europe and serve as an inspiration for similar work on other continents. In a divided Europe, this is also a sign of hope that collaboration and cooperation are both possible and deliver results.

Lastly, I hope that the data will serve as a baseline for conservation of fungi on a larger scale than that of country or region, and that it will show politicians that fungi, just like animals and plants, need protection.

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Book Announcement

Keys to Lichens of North America. Revised and expanded. By I.M. Brodo, 2016. Yale University Press, P.O. Box 209040, New Haven, CT 06520-9040, USA; <sales.press@yale.edu>. ISBN 978-0-300-19573-6; 424 p., 13 color + 33 black & white illustrations. Price US$ 29.95.

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Regional annotated mycobiotas new to the MYCOTAXON website

ABSTRACT — MYCOTAXON is pleased to announce four new species distribution lists to our 'web-list' page covering gasteroid agaricomycetes in Argentina (by Hernández Caffot et al.), *Hymenochaetaceae* in Northeast Brazil (by Lira et al,), corticioid fungi in the Canary Islands (by Beltrán-Tejera & al.), and lichenicolous fungi in Burdur Province (by Yazici & Etayo). This brings to 120 the number of free access mycobiotas now available on the MYCOTAXON website: http://www.mycotaxon.com/resources/weblists.html

SOUTH AMERICA

Argentina

MARÍA L. HERNÁNDEZ CAFFOT, XIMENA A. BROIERO, MARÍA E. FERNÁNDEZ, LEDA SILVERA RUIZ, ESTEBAN M. CRESPO & EDUARDO R. NOUHRA. Gasteroid mycobiota (*Agaricales, Geastrales* and *Phallales*) from Espinal forests in Argentina. 20 pp.

ABSTRACT—Sampling and analysis of gasteroid agaricomycete species (*Phallomycetidae* and *Agaricomycetidae*) associated with relicts of native Espinal forests in the southeast region of Córdoba, Argentina, have identified twenty-nine species in fourteen genera: *Bovista* (4), *Calvatia* (2), *Cyathus* (1), *Disciseda* (4), *Geastrum* (7), *Itajahya* (1), *Lycoperdon* (2), *Lysurus* (2), *Morganella* (1), *Mycenastrum* (1), *Myriostoma* (1), *Sphaerobolus* (1), *Tulostoma* (1), and *Vascellum* (1). The gasteroid species from the sampled Espinal forests showed an overall similarity with those recorded from neighboring phytogeographic regions; however, a new species of *Lysurus* was found and is briefly described, and *Bovista coprophila* is a new record for Argentina.

Brazil

CARLA R. S. DE LIRA, GEORGEA S. NOGUEIRA-MELO, LEIF RYVARDEN & TATIANA B. GIBERTONI. A checklist of *Hymenochaetaceae* from Northeast Brazil. 9 pp.

ABSTRACT—This study is an update of the available data about species of *Hymenochaetaceae* from Northeast Brazil. This revision is based on literature and herbaria revision besides recent field trips in the Atlantic Rain Forest and Caatinga biomes. Currently, there are 64 species and 12 genera of *Hymenochaetaceae* in Brazil. Among them, *Coltricia fragilissima* is reported for the first time in this country, while eight species are new to the Northeast region, 11 to the Brazilian semiarid and 22 to the Northeastern States.

Europe

Portugal & Spain (Iberian Peninsula)

Esperanza Beltrán-Tejera, J. Laura Rodríguez-Armas, M. Teresa Telleria, Margarita Dueñas, Ireneia Melo, Isabel Salcedo & J. Cardoso. Corticioid fungi of the western Canary Islands. Chorological additions. 74 pp.

Abstract—The diversity of corticioid fungi from the western Canary Islands is presented. Almost 200 species were found in 32 sites, including 63 species reported for the first time from the archipelago. Seventy-six genera are represented; the most common genera are *Hyphodontia, Trechispora, Peniophora, Botryobasidium, Hyphoderma, Tubulicrinis* and *Sistotrema*. Half of the genera are represented by a single species. Samples were collected from 32 plant species of which 22 are endemic to the Macaronesian bioregion. Although found in just 9 pine-dominated forest sites, *Pinus canariensis* was host to the most collections and species.

Mid-East

Turkey

Kenan Yazici & Javier Etayo. The lichenicolous fungi of Burdur province (Turkey). 9 pp.

Abstract — In the course of studies of lichens and bryophytes of Burdur province, Turkey, 42 species of lichenicolous fungi belonging to 20 genera in *Ascomycota* and *Basidiomycota* have been identified. Three of them, *Dactylospora parellaria, Didymellopsis perigena*, and *Zwackhiomacromyces constrictocarpus*, are new to Turkey in particular and Asia in general; short descriptions and distributional data are presented. In addition, the Turkish material of *Z. constrictocarpus,* hitherto only known from the type collection in Spain, represents the second record of this species.

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Nomenclatural novelties and typifications proposed in Mycotaxon 130(4)

Acaulospora reducta Oehl, B.T. Goto & C.M.R. Pereira [MB812332], p. 986

Codinaea aquatica R.F. Castañeda, M.S. Oliveira & Malosso [MB812851], p. 1046

Codinaea tropicalis (Bhat & W.B. Kendr.) R.F. Castañeda & Malosso [MB812854], p. 1048

Dictyosporium amoenum C.R. Silva, Gusmão & R.F. Castañeda [MB812865], p. 1126

Entyloma scandicis K.G. Savchenko, Carris, Castl., Heluta, Wasser & Nevo [MB811390], p. 1067

Hymenoscyphus aurantiacus H.D. Zheng & W.Y. Zhuang [FN570094], p. 1026

Hymenoscyphus macrodiscus H.D. Zheng & W.Y. Zhuang [FN570095], p. 1028

Hymenoscyphus qinghaiensis H.D. Zheng & W.Y. Zhuang [FN570096], p. 1030

Hymenoscyphus tetrasporus H.D. Zheng & W.Y. Zhuang [FN570098], p. 1031

Hymenoscyphus yui H.D. Zheng & W.Y. Zhuang [FN570099], p. 1033

Kroswia epispora H.J. Liu & Chao Li [MB811551], p. 952

Lecanicillium uredinophilum M.J. Park, S.B. Hong & H.D. Shin [MB814832], p. 1002

Matsushimiella paraensis J.S. Monteiro, Gusmão & R.F. Castañeda [MB810689], p. viii [validation of *Matsushimiella paraensis* J.S. Monteiro, Gusmão & R.F. Castañeda, Mycotaxon 130: 312 (2015), nom. inval., ICN (2012) Art. 42.1]

Neoalbatrellus subcaeruleoporus Audet & B.S. Luther [MB801480], p. 1194

Phoma candelariellae Z. Kocakaya & Halıcı [MB812271], p. 1186

Phyllachora jianfengensis Na Liu & M.Z. Li [FN570152], p. 1040

Pouzarella alissae Largent & Bergemann [MB812300], p. 1158

Pyramidospora quadricellularis M.S. Oliveira, Malosso & R.F. Castañeda [MB812756], p. 973

Synchaetomella aquatica Fiuza, Gusmão & R.F. Castañeda [MB812900], p. 1136

Terriera fici Y.R. Lin & Yuan Wu [MB812746], p. 1112

Tetrolylea Cantillo, R.F. Castañeda & Gusmão [MB812409], p. 978

Tetrolylea pleiomorpha Cantillo, R.F. Castañeda & Gusmão [MB812410], p. 978

Trichoconis foliicola S.S. Silva, Gusmão & R.F. Castañeda [MB812636], p. 1052

Tuber petrophilum Milenković, P. Jovan., Grebenc, Ivančević & Marković [MB812245], p. 1143

bad taxonomy

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